Original Article

Effect of Bupivacaine and Combination with Dexmedetomidine and Dexamethasone on Mice Neural Apoptosis

Mohammadreza Moshari[1], Ali Dabbagh[1], Mastaneh Dahi[1], Maryam Vosoughian[1], Behnam Hosseini[1], Fereshteh Baghizadeh[1], Seyed Mohammad Seyed-Alshohadaei[*]

Abstract

Background: Numerous studies have shown the neurotoxicity of anesthetic substances in different age groups. This toxicity is often associated with damage or apoptosis of nerve cells that can lead to various diseases, including Alzheimer's, behavioral changes and transient and even persistent cognitive changes. In this study, it was attempted to evaluate the cytotoxic conditions following the use of three common anesthetic drugs (bupivacaine, dexmedetomidine and dexamethasone) by providing a suitable substrate.

Methods and Materials: Mice (*Mus musculus*) with the same weight (22 to 30 gr) were used for assessment of neurotoxicity in Bupivacaine, Dexmedetomidine and Dexamethasone. Unilateral femoral nerve injections were done; animals were randomly divided into four groups: control, bupivacaine alone, "bupivacaine + dexmedetomidine" and "bupivacaine + dexamethasone". After 24 hours, the mice were sacrificed and the femoral nerve removed. Hematoxylin-eosin tissue staining was used to evaluate changes in the effects of the drugs, and nerve samples were extracted to assess the expression of TLR4 and caspase3. Protein expression level was checked between different groups using Western blot technique.

Results: The bupivacaine + dexamethasone group showed better outcomes in terms of cytotoxicity than bupivacaine + dexmedetomidine (p=0.568); also, bupivacaine + dexamethasone reduced neurotoxicity risk (P=0.431).

Conclusion: Bupivacaine+dexamethasone leeds to better outcomes in terms of neurotoxicity compared with bupivacaine+dexmedetomidine.

Keywords: Bupivacaine, Dexmedetomidine, Dexamethasone, Toll Like Receptor, Glyceraldehyde 3-phosphate dehydrogenase, Cysteine-aspartic acid protease

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

Corresponding Author: Seyed Mohammad Seyed-Alshohadaei, Anesthesiology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran. Tel: (+98) 21 22432572
Email: Sina.seyed80@gmail.com

Please cite this article as: Moshari MR, Dabbagh A, Dahi M, Vosoughian M, Hosseini B, Baghizadeh F, Seyed-Alshohadaei SM. Effect of Bupivacaine and Combination with Dexmedetomidine and Dexamethasone on Mice Neural Apoptosis. J Cell Mol Anesth. 2019;4(3):69-74.

Introduction

High-risk complications such as death, myocardial infarction, and pulmonary embolism, and other complications, such as postoperative nausea and

vomiting, are often an integral part of all anesthesia procedures (1). Numerous studies have challenged the neurotoxicity risks of anesthetic agents in animal studies which might lead to neurological cell damage; including Alzheimer's disease, behavioral and cognitive changes (2). The most important receptors involved in the pathway of brain development during anesthetic drug exposure are N-Methyl-D-Aspartate (NMDA) and Gamma-Aminobutyric Acid (GABA) (3-5). Anesthetic drugs that impair these receptors drive the cellular pathway of neuro-apoptosis. Given the limitations of the cellular repair in central nervous system, it is mandatory to reduce this risk (6).

Bupivacaine is one of the amide anesthetics used in neural block and neuraxial anesthesia that acts mainly through the sodium channel block with varying reports of effects on neurologic tissue cells (7-9). This drug has been shown to induce apoptotic effects in various studies (10). Amongst them, the effects on Schwann cells are one of the most prominent effects (11). Dexmedetomidine is an alpha-2 agonist that is recently used in a number of patient groups (12). Many studies show that the drug alone has safer profile regarding neurotoxicity issues (13). So that concomitant use with isoflurane reduces the rate of neuronal cell death (14). The majority of the studies have claimed this protective effect is due to the issue that it does not affect the GABA or NMDA receptor pathway (15). Dexamethasone is a type of corticosteroid medication (16). Corticosteroids have also been reported to have anti-apoptotic effects (17).

Toll-like receptors are found on the surface of dendritic cells, macrophages, neutrophils, mucosal cells, and other innate immune cells and identify their own agents from other alien cells (18). Meanwhile, TLR4 is originally introduced as an endotoxin detector (LPS) and a cellular content-related injury marker such as DNA, RNA, and the cytoplasm (19). Previous studies have demonstrated the role of this protein in neural cell damage and the formation of oxygen free radicals. Therefore, TLR4 level assay can be a determining factor in cytotoxicity (20).

The caspase3 protein is a member of the cysteine-aspartic acid protease (caspase) family (21). Sequential activation of caspases plays a central role in the execution phase of cell apoptosis (22).

This study was designed to investigate the neurotoxic effects of bupivacaine when used as local anesthetic, alone or with either dexamethasone or dexmedetomidine drugs after injection into the rat femoral nerve.

Methods

Mice (*Mus musculus*) with a similar weight (22 to 30 gr) used for neurotoxicity evaluation of Bupivacaine, Dexmedetomidine, and Dexamethasone. Mice were kept and used according to the companion animal ethics (23).

Injections into the femoral nerve were performed unilaterally and rats were randomly divided into four groups (in each group, four mice) of control (Ketamine 300 µl+ Xylazine 100 µl), bupivacaine alone (Ketamine 300µl+ Xylazine 100µl +bupivacaine 2mg/kg), bupivacaine in combination with dexmedetomidine (Ketamine 300 µl+ Xylazine 100 µl +bupivacaine 2 mg/kg +Dex 1 mg/kg) and in group 4, bupivacaine was injected with dexamethasone (Ketamine 300 µl+ Xylazine 100 µl +bupivacaine 2 mg/kg +Dexa 5 mg/kg).

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

The protein then extracted by radioimmunoprecipitation assay buffer (RIPA buffer). The expression levels of the proteins evaluated by electrophoresis and western blotting. At this stage, 20-40 µg of protein used for Western blotting. ImageJ software used to detect and analyze images from Western blotting.

This study 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.

Results

The expression of TLR4 and caspase3 (as contributing factors to apoptosis induction and toxicity) were compared with GAPDH (as a house-keeping gene and baseline) in four different groups. As

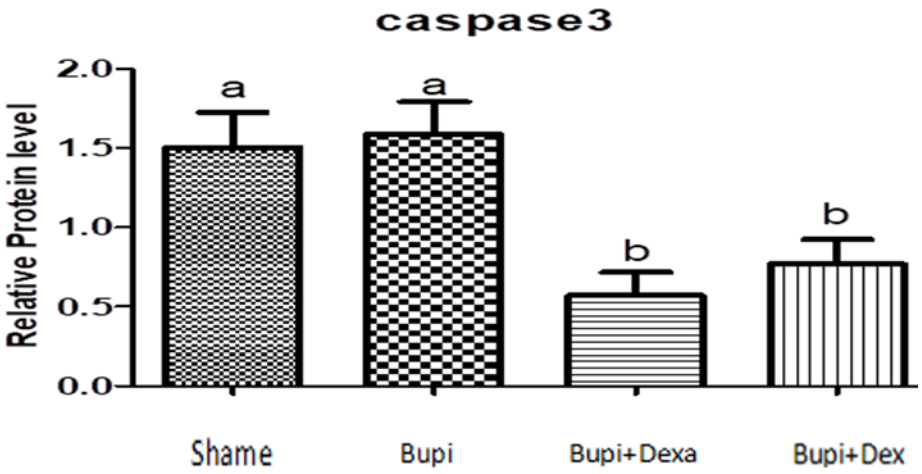

Figure 1. Diagram (P. Value) of caspase3 expression compared to GAPDH in rat femoral nerve (24 h after injection) in four groups (control, bupivacaine, bupivacaine + dexamethasone and bupivacaine + dexmedetomidine).

can be seen in fig 1, the expression index of caspase 3 biomarker was presented based on the amount and thickness of the band produced on SDS gel following Western blot technique (Figure 1).

Figure 2 shows the electrophoresis results of the proteins extracted on the gel. This figure presents the qualitative range of affinity of the target protein (caspase3 marker) to the standard GAPDH index.

Figure 3 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 4 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

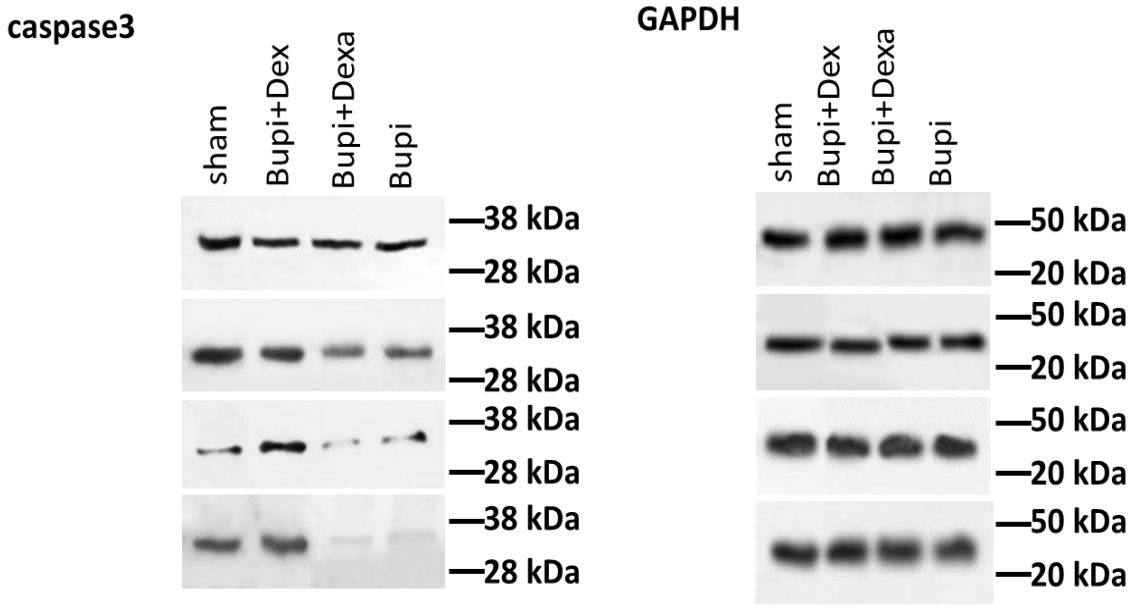

Figure 2. Qualitative comparison of caspase3 protein expression in rat femoral nerve for four groups (control, bupivacaine, bupivacaine + dexamethasone and bupivacaine + dexmedetomidine) compared to standard GAPDH index protein.

standard GAPDH index. under the combined use of bupivacaine +

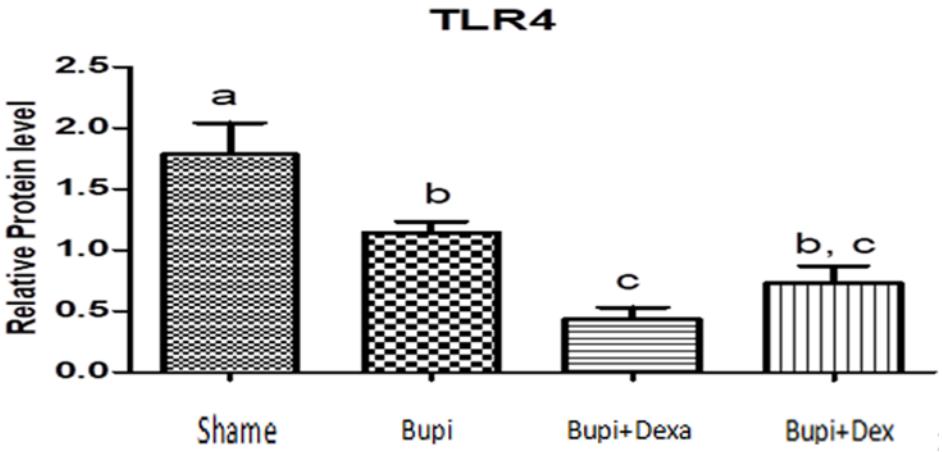

Figure 3. Diagram (P. Value) of TLR4 expression compared to GAPDH in rat femoral nerve (24 h after injection) in four groups (control, bupivacaine, bupivacaine + dexamethasone and bupivacaine + dexmedetomidine).

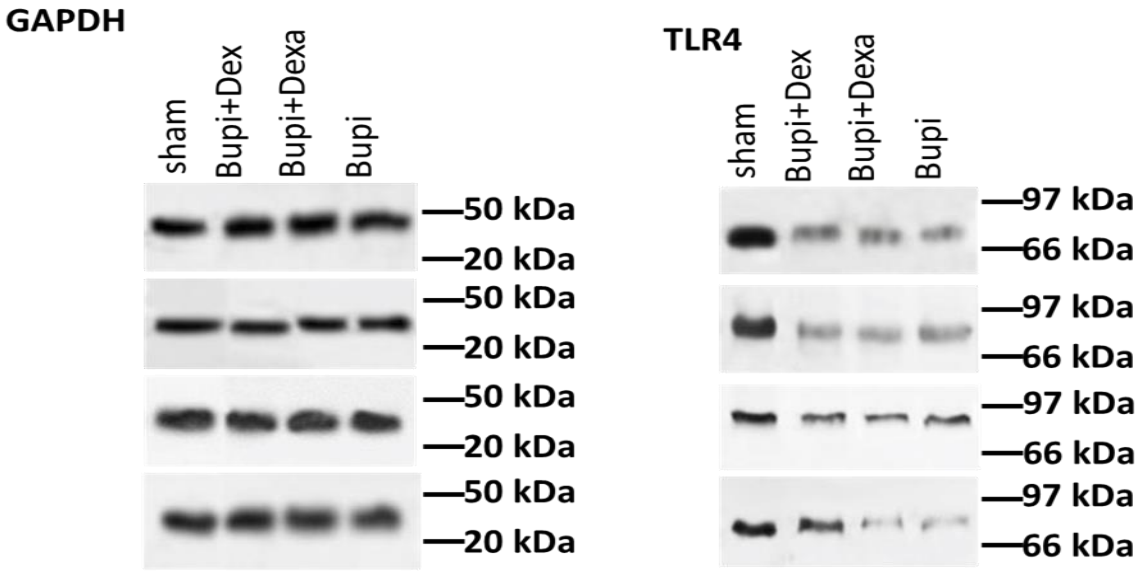

Figure 4. Qualitative comparison of TLR4 protein expression in rat femoral nerve for four groups (control, bupivacaine, bupivacaine + dexamethasone and bupivacaine + dexmedetomidine) compared to standard GAPDH index protein.

Discussion

The results of the present study emphasized the neuro-cytotoxic damage of these drugs. For this purpose, two molecular markers of apoptosis initiation (TLR4 and caspase3) evaluated as an indicator of cellular damage induced by the use of drugs used on rat femoral nerves (24 h after injection). Thus, the expression of both the TLR4 and caspase3 markers dexmedetomidine and bupivacaine + dexamethasone has the low affinity with the baseline index (GAPDH). Simply put, the use of bupivacaine in combination with two other drugs (dexmedetomidine and dexamethasone) has caused less damage to mouse neurons. However, the combination of bupivacaine with dexamethasone (p=0.568 and p=0.431) showed relatively better conditions in terms of cytotoxicity than the combination of bupivacaine with dexmedetomidine (p=0.768 and p=0.725) (Figs. 1 and

3).

The damages that occur following the use of all kinds of anesthetic materials are caused by activation of microglia and the presence of astrocytes and by the involvement of neurons. Previously, the association between inflammation in the nervous system and neurodegenerative diseases has been proven to increase the risk of Alzheimer's disease by pro-inflammatory markers. This process is likely to cause brain damage after transient ischemia, either primary or secondary.

Gözil R et al. in 2002 introduced bupivacaine as the drug of choice for neural obstruction. According to their observations, this drug, while less damaging, accelerates the regeneration process (24). In 2016, O'Connor and colleagues reported that dexamethasone alone (at a dose of 10 µm or higher) had protective effects on neurons. Therefore, concurrent use of adrenergic substances such as dexmedetomidine may reduce neuronal cell damage (25). Wu et al. (2014) published a study on the synergistic effect of dexmedetomidine and ropivacaine on the inhibition of neuron-astrocyte activity. According to their study, concomitant use of both drugs increased the quality of anesthesia and decreased astrocyte activity (26). As a result, they suggested that the combination of the two drugs would improve conditions to improve the effect of anesthesia and reduce inflammation in the central nervous system.

Conclusion

Concomitant administration of bupivacaine with dexmedetomidine and dexamethasone reduces bupivacaine neurotoxicity. This is while, concomitant use of bupivacaine+dexamethasone has led to better conditions than concomitant use of bupivacaine+dexmedetomidine in reducing neurotoxicity.

Conflicts of Interest

The authors declare that they have no conflict of interest.

References

1. Kessler J, Marhofer P, Hopkins PM, Hollmann MW. Peripheral regional anaesthesia and outcome: lessons learned from the last 10 years. Br J Anaesth. 2015;114(5):728-45.

2. Pöpping DM, Elia N, Van Aken HK, Marret E, Schug SA, Kranke P, Wenk M, Tramèr MR. Impact of epidural analgesia on mortality and morbidity after surgery: systematic review and meta-analysis of randomized controlled trials. Ann Surg. 2014;259(6):1056-67.

3. Katayama H. Anti-interleukin-17A and anti-interleukin-23 antibodies may be effective against Alzheimer's disease: Role of neutrophils in the pathogenesis. Brain Behav. 2020 Jan;10(1):e01504.

4. Cameron B, Landreth GE. Inflammation, microglia, and Alzheimer's disease. Neurobiol Dis. 2010;37(3):503-9.

5. Heneka MT, O'Bannon MK. Inflammatory processes in Alzheimer's disease. J Neuroimmunol. 2007;184(1-2):69-91.

6. Srivastava A, Kumar V, Pandey A, Jahan S, Kumar D, Raj purohit CS, Singh S, Khanna VK, Pant AB. Adoptive autophagy activation: a much-needed remedy against chemical induced neurotoxicity/developmental neurotoxicity. Mol neurobiol. 2017; 54(3): 1797-807.

7. Weinberg GL, Ripper R, Murphy P, Edelman LB, Hoffman W, Strichartz G, Feinstein DL. Lipid infusion accelerates removal of bupivacaine and recovery from bupivacaine toxicity in the isolated rat heart. Reg Anesth Pain Med. 2006 Jul-Aug;31(4):296-303..

8. Heinonen JA, Schramko AA, Skrifvars MB, Litonius E, Backman JT, Mervaala E, Rosenberg PH. The effects of intravenous lipid emulsion on hemodynamic recovery and myocardial cell mitochondrial function after bupivacaine toxicity in anesthetized pigs. Hum Exp Toxicol. 2017;36(4):365-75.

9. Bourne E, Wright C, Royse C. A review of local anesthetic cardiotoxicity and treatment with lipid emulsion. Local Reg Anesth. 2010;3:11-9.

10. Xuan W, Zhao H, Hankin J, Chen L, Yao S, Ma D. Local anesthetic bupivacaine induced ovarian and prostate cancer apoptotic cell death and underlying mechanisms in vitro. Sci Rep. 2016; 6:26277.

11. An K, Elkassabany NM, Liu J. Dexamethasone as adjuvant to bupivacaine prolongs the duration of thermal antinociception and prevents bupivacaine-induced rebound hyperalgesia via regional mechanism in a mouse sciatic nerve block model. PLoS One. 2015;10(4): e0123459.

12. Green M, Chatterjee D, Meyers M. High dose dexmedetomidine and ketamine for managing difficult pediatric airways. J Anesth Clin Res. 2016;7: 652.

13. Yang W, Kong LS, Zhu XX, Wang RX, Liu Y, Chen LR. Effect of dexmedetomidine on postoperative cognitive dysfunction and inflammation in patients after general anaesthesia: A PRISMA-compliant systematic review and meta-analysis. Medicine (Baltimore). 2019;98(18):e15383.

14. Li Y, Zeng M, Chen W, Liu C, Wang F, Han X, Zuo Z, Peng S. Dexmedetomidine reduces isoflurane-induced neuroapoptosis partly by preserving PI3K/Akt pathway in the hippocampus of neonatal rats. PLoS One. 2014 Apr 17;9(4):e93639.

15. Lin S, Zhou G, Shao W, Fu Z. Impact of dexmedetomidine on amino acid contents and the cerebral ultrastructure of rats with cerebral ischemia-reperfusion injury. Acta Cir Bras. 2017;32(6):459-66.

16. Go SI, Koo DH, Kim ST, Song HN, Kim RB, Jang JS, Oh SY, Lee KH, Lee SI, Kim SG, Park LC. Antiemetic Corticosteroid Rotation from Dexamethasone to Methylprednisolone to Prevent

Dexamethasone- Induced Hiccup in Cancer Patients Treated with Chemotherapy: A Randomized, Single- Blind, Crossover Phase III Trial. Oncologist. 2017;22(11):1354-61.
17. Zidan A, Hedya SE, Elfeky DM, Abdin AA. The possible anti-apoptotic and antioxidant effects of acetyl l-carnitine as an add-on therapy on a relapsing-remitting model of experimental autoimmune encephalomyelitis in rats. Biomed Pharmacother. 2018;103:1302-11.
18. Takeda K, Akira S. Toll- like receptors. Curr Protoc Immunol. 2015;109:14.12.1-14.12.10.
19. Eleftheriadis T, Pissas G, Liakopoulos V, Stefanidis I. Cytochrome c as a potentially clinical useful marker of mitochondrial and cellular damage. Front Immunol. 2016;7:279.
20. Lu Y, Liu J, Liu Y, Qin Y, Luo Q, Wang Q, Duan H. TLR4 plays a crucial role in MSC-induced inhibition of NK cell function. Biochem Biophys Res Commun. 2015;464(2):541-7.
21. Fan TJ, Han LH, Cong RS, Liang J. Caspase family proteases and apoptosis. Acta Biochim Biophys Sin (Shanghai). 2005;37(11):719-27.
22. Porter AG, Jänicke RU. Emerging roles of caspase-3 in apoptosis. Cell Death Differ. 1999;6(2):99-104.
23. Springer S, Sandøe P, Bøker Lund T, Grimm H. "Patients' interests first, but … "¯Austrian Veterinarians' Attitudes to Moral Challenges in Modern Small Animal Practice. Animals (Basel). 2019;9(5).
24. Gonca E, Çatlı D. The Effects of Lidocaine with Epinephrine on Bupivacaine-Induced Cardiotoxicity. Turk J Anaesthesiol Reanim. 2018;46(6):447-452.
25. O'Connor SD, Cabrera OH, Dougherty JD, Singh S, Swiney BS, Salinas-Contreras P, Farber NB, Noguchi KK. Dexmedetomidine protects against glucocorticoid induced progenitor cell apoptosis in neonatal mouse cerebellum. J Matern Fetal Neonatal Med. 2017 Sep;30(18):2156-2162.
26. Wu HH, Yin JB, Zhang T, Cui YY, Dong YL, Chen GZ, Wang W. Inhibiting spinal neuron-astrocytic activation correlates with synergistic analgesia of dexmedetomidine and ropivacaine. PLoS One. 2014;9(3):e92374.