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Comparison of histopathological effects of perineural administration of bupivacaine and bupivacaine-dexmedetomidine in rat sciatic nerve

Elham Memari[a], Mohammad-Ali Hosseinian[b], Ali Mirkheshti[a], Ali Arhami-Dolatabadi[g], Mojtaba Mirabotalebi[a], Mohsen Khandaghy[a], Yahya Daneshbod[c], Leila Alizadeh[e], Sadegh Shirian[d,e,f,*]

[a] Anesthesiology Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[b] Department of General Surgery, Shahid Beheshti University of Medical Science, College of Medicine, Emam Hosein Hospital, Tehran, Iran
[c] Shiraz Molecular Pathology Research Center, Dr. Daneshbod Pathology Laboratory, Shiraz, Iran
[d] Department of Pathology, School of Veterinary Medicine, Shahrekord University, Shahrekord, Iran
[e] Shefa Neuroscience Research Center, Khatam-Al-Anbia Hospital, Tehran, Iran
[f] Brain and Spinal Cord Injury Research Center, Neuroscience Institute, Tehran University of Medical Sciences, Tehran, Iran
[g] Department of Emergency Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran

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ABSTRACT

Injection of a variety of drugs such as local anesthetics (LAs) for peripheral nerve block has been shown to cause damage to peripheral nerves. Bupivacaine is a local anesthetic widely used in surgical procedures. The aim of this study was to evaluate the neurotoxicity of LAs including Bupivacaine and dexmedetomidine (DEX)-Bupivacaine on sciatic nerve tissue at histopathological level. In addition, we investigated whether perineural administration of DEX can attenuate Bupivacaine-induced neurotoxicity. Twenty adult Sprague Dawley rats received unilateral sciatic nerve blocks with either 0.2 ml of 0.5% bupivacaine (n = 8) or 0.5% bupivacaine plus 0.005% DEX (n = 8) or normal saline (0.9%, as control group) (n = 4) in the left hind extremity. Sciatic nerves were harvested at 14 days post-injection and analyzed for nerve damage using ultrastructure and histopathologic analysis. Histopathology of sciatic nerve at day 14 post-injection showed a variable degree of neuronal injury associated with perineural inflammation in each treatment group and was classified as none or mild, intermediate or severe. Administration of both LAs resulted in a significant decrease in the total number of myelinated fibers per nerve (95% CI for group difference: Bupivacaine, $P = 0.001$, DEX-Bupivacaine, $P = 0.036$) compared to the saline control group. Animals that received these perineural local anesthetics (LAs) injections showed increased severity of injury compared to the control group. Animals in the DEX-Bupivacaine group had higher perineural inflammation and nerve damage than those of the saline control group and less than those of the Bupivacaine group at day 14 post-injection. Quantitatively, average total nerve fiber per nerve and average myelinated nerve fiber density in the injured region of the Bupivacaine-treated group was less than that of the DEX-Bupivacaine-treated group. LAs injection into the nerve causes peripheral nerve damage and remains an important clinical danger. Bupivacaine is associated with considerable histopathological changes, including edema of the perineurium and myelin degeneration with Wallerian degeneration, when injected perineurally. Perineural DEX added to a clinical concentration of bupivacaine attenuates the Bupivacaine-induced injuries.

1. Introduction

Injection of a variety of drugs such as local anesthetics (LAs) to block peripheral nerve has been shown to cause damage to the peripheral nerves (Auroy et al., 2002; Hertl et al., 1998; Whitlock et al., 2010). This damage is results from needling or toxicity of the medication used (Farber et al., 2013). As a result of intraneural injection of LAs some complications, such as loss of sensation,

* Corresponding author at: Department of Pathology, School of Veterinary Medicine, Shahrekord University, Shahrekord, Iran.
E-mail addresses: s-shirian@razi.tums.ac.ir, s-shirian@razi.tums.ac.ir, http://mailto:s-shirian@razi.tums.ac.ir (S. Shirian).

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motor function, pain, and causalgia have been also reported (Farber et al., 2013). Local anesthetics such as lidocaine, its derivatives tetracaine and procaine lead to severe nerve damage manifested as splitting of the myelin lamellae followed by widespread axonal and myelin degeneration (Hunter et al., 2007). Conversely, Marcaine which is marketed under the brand name Bupivacaine, has been associated with neuronal damage when injected directly into the peripheral nerve (Farber et al., 2013; Hertl et al., 1998). The combined adjuvants; clonidine, buprenorphine, and dexamethasone do not appear to alter LAs neurotoxicity (Knight et al., 2015). However, there is increasing evidence of DEX-related organ protective effects against ischemic and hypoxic injury such as neuroprotection (Panzer et al., 2009). Several experimental studies have suggested that the intraneural injection of LAs is neurotoxic (Farber et al., 2013; Hertl et al., 1998). To date, there are no clinical or experimental reports of DEX-induced neuronal injury. In addition, it has actually been demonstrated that DEX attenuates the neurotoxicity of bupivacaine at the cellular level (Brummett et al., 2008; Malet et al., 2015). To the best of our knowledge, no studies have explored the effect of DEX on attenuation of neural injury resulting from LAs at the histopathological level. The aim of this study was to evaluate the neurotoxicity of LAs, including Bupivacaine and dexmedetomidine (DEX)-Bupivacaine, on sciatic nerve tissue at the histopathological level. Therefore, a total 20 Sprague-Dawley rats were randomly divided into three groups. The first group was selected as the control group which received normal saline (n = 4), followed by the second and third groups which were injected with Bupivacaine (n = 8) and mixed Bupivacaine and DEX (n = 8), respectively. Sciatic nerves were harvested at 14 days post-injection and analyzed for nerve damage using ultrastructure and histopathologic analysis. We also investigated whether perineural administration of DEX can attenuate Bupivacaine-induced neurotoxicity.

2. Material and methods

This study was approved by the Medical University of Shahid Beheshti Committee for the Use and Care of Animals. All procedures were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals and the Guidelines for the Care and Use of Mammals in Neuroscience and Behavioral Research.

Twenty Sprague-Dawley rats (initial body weight 220 ± 20 g) were obtained from the breeding colony of the animal house of Shahid Beheshti Medical University. They had free access to commercial chow and tap water, in a temperature-controlled room (23 ± 1 °C) with a 12 h light–dark cycle. Animals were randomly divided into three groups, corresponding to injected drug. The first group was selected as the control group (n = 4) which received normal saline (0.9%), followed by the second and third groups which were injected with Bupivacaine (0.5%, AstraZeneca, Australia) (n = 8) and mixed Bupivacaine (0.5%, AstraZeneca, Australia) and DEX (0.005%, Hospira, USA) (n = 8), respectively. A single treatment of all drugs was administered perineurally in total volume of 0.2 ml to the sciatic nerve. All drugs were administered perineurally (a single treatment) in total volume of 0.2 ml to the sciatic nerve.

Animals were anesthetized by intraperitoneal administration of a mixture of 10 mg/kg ketamine and 10 mg/kg xylazine (Fort Dodge Animal Health, Fort Dodge, IA). The sciatic nerve of the left hind extremity was exposed aseptically under an operating microscope (Wild Heerbrugg, Heerbrugg, Switzerland). Following the dissection, the sciatic nerve was clearly identified at a point proximal its bifurcation and used as a reproducible injection site. The perineural injections were performed using an insulin syringe fitted with a 29-gauge × 1/2″ needle (Terumo Medical Co., Elkton, MD). To minimize the variation resulting from the injury induced by needling, the same method of injection was blindly used for every animal by the same operator.

Under direct vision, all rats were injected with a total volume of 0.2 ml of drug per injection into the perineural space below the clear fascia covering the nerve and proximal to the bifurcation of the sciatic nerve. The injection site was marked with a single 10-0 nylon microsuture. Muscle and skin were reapproximated with 6-0 Vicryl (Ethicon Inc., Somerville, NJ) and 4-0 nylon, respectively.

At the 2-week end point, 10 mm section of the sciatic nerve consist of both proximal and distal to the injection site was harvested to evaluate the extent of nerve injury using light microscopy and electron microscopy. ensuring that a 10 mm section, both proximal and distal to the injection site, was removed en bloc.

The animals were euthanized with intracardiac injection of 200 mg/kg sodium pentobarbital and the harvested nerves were promptly placed in 4% glutaraldehyde solution at 4 °C for 4 h to be prepared for ultrastructure analysis. The distal sciatic nerve segments were then post-fixed with 1% osmium tetroxide, embedded in resin, and sectioned into approximately 1 μm slices. The specimens were then stained with toluidine blue and examined using light microscopy. A transmission electron microscope (Philips, USA) was used to examine the double stained ultrathin (60–90 nm) sections which were prepared using a LKB NOVA ultratome and stained with uranyl acetate and lead citrate.

The proximal sciatic nerve fixed in 10% neutral buffered formalin, processed routinely and embedded in paraffin wax, was sectioned into approximately 5 μm slices and stained by hematoxylin and eosin (HE). A Pathologist, blinded to the treatment groups, performed histopathological and ultrastructure analysis.

3. Statistical analysis

Data analyses were done using the SPSS 22.0 software package. All data are expressed as mean (±SD). After testing for normality of pairwise differences with Shapiro-Wilk normality test, the effect of Bupivacaine and DEX-Bupivacaine on fiber density was compared between groups using independent *t*-tests. A value of $P < 0.05$ was considered statistically significant.

4. Results

Review of the light and EM sections was conducted blindly by an expert pathologist under different magnifications and was recorded according to Table 1.

Histopathological examination of the sciatic nerve at 14 days post-injection showed a variable degree of neuronal injury associated with perineural inflammation in each treatment group and was classified as none or mild, intermediate, or severe (Table 1). Perineural injection of saline in control group animals resulted in normal histology and no damage was observed in three cases. However, one of the animals in this group showed intermediate injury. Nerves in the DEX-Bupivacaine group showed less perineural inflammation at two weeks post-injection compared to the Bupivacaine group. Inflammation was accompanied by infiltration with large numbers of lymphocytes surrounding the nerve. The DEX-Bupivacaine group had higher perineural inflammation than that of the saline control group and less than that of the Bupivacaine group at 2 weeks post-injection.

Most of the saline control group (3 cases) and DEX-Bupivacaine cases (5 cases) showed no histopathological nerve damage, including demyelination, perineural inflammation, Wallerian degeneration and proliferation of inflammatory cells or showed mild injury (Fig. 1A and B), while only one of the Bupivacaine cases