Potential neurotoxic effect of dexamethasone used as adjuvant to local anesthetics during peripheral nerve blockade

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ABSTRACT

Objectives: The use of peripheral nerve blocks for anesthesia and postoperative analgesia has increased significantly in recent years. Many additives to local anesthetics to prolong the duration of analgesia for peripheral nerve blocks have been studied. Dexamethasone has been studied as an effective adjuvant to prolong the analgesia duration of local anesthetics in peripheral nerve block. However, the route of action for dexamethasone and its potential neurotoxicity are still unclear. The aim of this study is to determine possible toxic effects of dexamethasone on peripheral nerve tissue and the dependence of these effects on the place of intraneural applications.

Methods: A rat sciatic nerve block model was used. The study was conducted in accordance with the principles of laboratory animal care and was approved by the Laboratory Animal Care and Use Committee. Fifty adult Wistar rats (300 g) both sexes were studied. After induction of general anesthesia, the sciatic nerve was exposed bilaterally. Sciatic nerves were randomly assigned by the method of sealed envelopes to receive: intraneural, intrafascicular 2 mL of lidocaine with dexamethasone (n=25), intraneural, extrafascicular injection 2 mL of lidocaine with dexamethasone (n=25), perineural 2 mL of lidocaine with dexamethasone (n=25) and perineural 2 mL of saline 0.9% (n=25). Injection pressure was continuously recorded using an in-line digital manometer. Increased injection pressure was used to distinguish intrafascicular from extrafascicular intraneural injections. After injection, the rats were awakened and subjected to serial neurologic examinations. Neurologic examination protocol was followed to determine proprioception by tactile place response, motor function by extensor postural thrust and nociception by withdrawal reflex. On day 3 of the experiment, the animals were sacrificed and the neural tissue histologically examined.

Results: Intraneural injections (intrafascicular and extrafascicular) of lidocaine in combination with dexamethasone caused neurological deficits and severe pathohistological damage to nerve fibers. All perineural injections (independent of the tested solution), combined with low injection pressure showed a uniform change, with minimal histological deviation of the normal structure of the nerve fiber.

Conclusion: When applied intraneurally dexamethasone in combination with lidocaine caused nerve fiber damage. However, future studies are required to elucidate the most effective route and optimum dosing range for dexamethasone’s use in this field.

Keywords: dexamethasone, local anesthetic, peripheral nerve blockade

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INTRODUCTION

The use of local anesthetic peripheral nerve blocks for surgical anesthesia and postoperative pain management has increased significantly [1]. Single-shot peripheral nerve blocks as an alternative to general anesthesia and an opioid-sparing analgesic have become a portion of standard anesthesia practice throughout the world. Commercially available local anesthetics have a limited duration of analgesia that frequently leaves patients complaining of pain for the first time during their first postoperative night when they are likely most vulnerable. While there are longer acting formulations and new concepts on the horizon, there are limits to what local anesthetics alone can provide [2]. Analgesia is rarely maintained for more than 2-4 hours after injection application. The need for prolonged pain relief led to the use of continuous infusion of local anesthetics through the catheter and manufacture of long-acting local anesthetics such as bupivacaine, ropivacaine and levobupivacaine [3]. Numerous recent randomized controlled trials and meta-analyses have examined the pros and cons of the use of various individual adjuvants thought to potentially enhance local anesthetic peripheral nerve blockade. More recent approach to achieve long lasting effect of local anesthesia is the use of adjuvants to local anesthetics, such as epinephrine, clonidine [4,5], opioids [6,7], ketamine [8,9], midazolam [10]. Because of limited efficacy and questionable toxicity of agents listed, some researchers have begun to carry out an evaluation of glucocorticoids as the addi-
tion of local anesthetics in regional anesthesia. They are known for its anti-inflammatory, immunosuppressive and anti-emetic capacity, which is evaluated in small number of preclinical [11-13] and clinical trials [14-16]. Although corticosteroids are often administered by injection in the vicinity of peripheral nerves, there is no experimental study which defined the possible neurotoxic effects of these agents. The aim of this study is to determine possible toxic effects of dexamethasone on peripheral nerve tissue and the dependence of these effects on the place of intraneural applications.

**MATERIAL AND METHODS**

**Animals**

Fifty Wistar rats, both sexes (300-350 g, 3 months old) were used in this study. Animals were housed in central animal care facility. Food and water were provided ad libitum. Animals were monitored for appropriate post-surgical recovery. The study was conducted in accordance with the Principles of Laboratory Animal Care [17] and was approved by the Laboratory Animal Care and Use Committee of the Medical and Veterinary Schools of the University of Sarajevo. All study procedures were approved by the Ethical Committee of Sarajevo University School of Medicine.

**Experimental design**

On the day 1 of experiment, the rats were anesthetized with pentobarbital sodium (50 mg per kilogram of body weight) administrated intraperitoneally. Thereafter, by using an aseptic technique for survival surgery, the sciatic nerves (n=100) were exposed bilaterally through a gluteal muscle-splitting incision. Sciatic surgery, the sciatic nerves (n=100) were exposed bilaterally through a gluteal muscle-splitting incision. Sciatic nerves were randomly assigned by the method of sealed envelopes to receive:

1. **Intraneural, intrafascicular group** – 2 mL of lidocaine with dexamethasone #25 injection
2. **Intraneural, extrafascicular injection** - 2 mL of lidocaine with dexamethasone #25 injection
3. **Perineural group** - 2 mL of lidocaine with dexamethasone #25 injection
4. **Perineural group** – 2 mL of saline 0.9% #25 injection

Under the direct visual control, the needle (Becton Dickinson Microlance 000800), with the diameter 27 G (gauge), 12.7 mm long cut, under the angle of 45°, in the direction distal-proximal was placed intraneural (subperineural) into sciatic nerve on one side, and then perineural (subepineural) into sciatic nerve on the other side of every examination groups. Using the automatic syringe charger (PHD2000; Harvard Apparatus, Holliston, MA), which regulates the volume and the speed of applied solution, in previously mentioned structures we applied 2 mL of 2% lidocaine with addition of 4mg/mL of dexamethasone in examination groups or saline in control groups, with the speed of 5mL/min. Injection pressure was continuously recorded using an inline digital manometer (BioBench). Increased injection pressure was used to distinguish intrafascicular from extrafascicular intraneural injections.

After injection, animals were allowed to wake up from anesthesia and were given a series of neurologic examinations according to Thalhammer [18]. Neurologic examination were performed hourly for the next 6 hour and daily for the next 3 days, and included assessment of proprioception, motor function and nociception. Proprioception was evaluated by testing postural reactions (tactile placement response – the rat was kept in a normal resting posture, toes of one foot were flexed with their dorsal part placed onto the supporting surface and the ability to reposition the toes were evaluated). The functional deficit was graded as: 0 – normal; 1 – slightly impaired; 2 – severely impaired; 3 – absent.

Block duration was defined as time which passes until the response returns to score 1.

Motor function was evaluated by measuring extensor postural thrust: the rat was held upright with the hind limb extended to that the body’s weight was supported by the distal metatarsus and the toes and the extensor postural thrust could be measured as the force applied to the digital balance, the force that resist contact of the platform balance by the heel. The reduction in the force, representing reduced extensor muscle tone, was considered as a deficit of motor function and expressed as percentage of the control force. Before applying lidocaine with dexamethasone, a test will be performed to evaluate the normal test value (NEPT). The value of the test before and after the application of lidocaine with dexamethasone (EEPT), will be included in the formula for calculating the percentage of motor deficit.

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\text{Percentage of motor deficit} = \frac{(\text{NEPT} - \text{EEPT})}{\text{NEPT}} \times 100
\]

The duration of the motor blockade was defined as the time required for recovery to 25% of motor deficit.

Nociception was evaluated by observing the withdrawal of the limb in response to noxious stimulation as: 4 – normal withdrawal reaction, rapid withdrawal of the paw, vocalization, bites the forceps; 3 – slower withdrawal reaction, slower withdrawal of the pinched extremity, vocalization, no attempts to bite the forceps; 2 – slow withdrawal reaction, no vocalization, no attempts to bite the forceps; 1 – barely perceptible withdrawal, no vocalization, no attempts to bite the forceps; 0 – no withdrawal, no vocalization, no attempts to bite the forceps.

The block duration was defined as time which passes until the response returns to score 3 (75 % of normal). The animals were euthanized 3 days after injection.
of the test solutions, and the specimens of the sciatic nerve on the block with the neighboring tissues were removed. The samples were fixed in formalin and paraffin followed by microtome sections and stained with hematoxylin and eosin method. Qualitative histological analysis of the samples was performed by pathologist blinded to the study groups, and included assessment of presence or absence of edema, fat drops, nerve fiber damage and inflammatory cells.

Statistical analysis

Statistical analysis was performed by using SPSS program, version 11.5. (SPSS, Inc., Chicago, IL, USA). One hundred sciatic nerves (50 rats) were required for the power of 0.80 to detect a significant difference in proportions of nerve injury between intraneural and perineural injections at $\alpha=0.05$ [19]. Rates of neurologic and histological injuries were compared between intraneural and perineural injection by McNemar’s test for paired proportions. Fisher’s exact test was used to compare injury rates during the intraneural injection, based on injected solution. P value of <0.05 was considered to be significant.

RESULTS

Neurologic outcome

After recovery from general anesthesia, sensory-motor sciatic blockade was evident in rats that received lidocaine and dexamethasone but not in rats who received saline. It has been found that all intraneural injections (intrafascicular and extrafascicular) of lidocaine in combination with dexamethasone resulted in deficit which lasted more than 24 hours, and neurological deficits were evidential at the end of experiment, after 3 days, which clearly shows that intraneural injection caused nerve damage (Figure 1,2,3).

On the contrary, perineural injections did not resulted with neurological sequel after the end of experiment. Furthermore, in most cases neurological deficit has withdrawn within first 24 hours of experiment (Figure 1,2,3).

Histopathological examination

All perineural injections (independent of the tested solution), combined with low injection pressure showed a uniform changes, with minimal histological deviation of the normal structure of the nerve fiber. Most of nerves had epineurium with preserved structure. However, it has been observed in some places hyperemic blood vessels and extravasation of erythrocytes, and no uniform distribution of collagen fibers. Perineurium was partly edematous with stratified lamellas. Central nerve fibers were preserved. Intrafascicular blood vessels showed no signs of hyperemia (Figure 4).

By contrast, pathological changes were significant in intraneural groups with lidocain in combination with dexamethasone. Intrafascicular injection groups marked cellular infiltration, subperineural edema and diffuse axonal swelling were apparent. Pathological conditions in the periphery of the fascicle were more prominent than in the central zone. Nerve fibers showed different degree of damage, enlarge of the volume, the disintegration of the myelin sheath, eccentrically positioned axons up to complete disintegration of the nerve fibers. Perineurium showed division of lamellas with its significant disintegration at the place of puncture and the loss of demarcation toward the surrounding perifascicular connective tissue and closest nerve fibers. Epineurium shows hyper cellularity of the
mononuclear inflammatory process type with an increased number of macrophages, lymphocytes, plasma cells, hyperemic blood vessels and bundles of collagen fibers with uneven tinctorial attributes (Figure 5).

**Discussion**

Local anesthetics are widely used for peripheral nerve blockade. While there are a wide range of procedures for which short acting local anesthesia is ideal, it is becoming increasingly clear that long duration blocks are useful, particularly in the perioperative setting [20,21]. One of several strategies to that have been employed to increase the duration of local anesthetic block of peripheral nerves is based on the use of adjuvants that, when used in combination with local anesthetics, can prolong the duration of action. Several perineural adjuvants have been studied with the goal of prolonging the duration of analgesia for a given local anesthetic dose. Furthermore, the efficacy of these adjuvants used in combination with local anesthetics has encouraged anesthesiologist to use them in the absence of local anesthetics, in the hope that an optimal combination of adjuvants might have modality selectivity (sensory instead of motor fiber block) [20,22]. From the patient care perspective, in the context of peripheral nerve damage after local anesthetic blockade, toxicity to neurons in culture should be considered a potentially relevant predictor, given that up to 3% of patients have complications that can persist up to 6 months after nerve blocks with local anesthetic [23,24]. Neurotoxicity of agents administered along with local anesthetics remains an important concern. Many of the adjuvants have not been fully scrutinized with rigorous research demonstrating their safety in children at various stages of development [25].

Dexamethasone is a synthetic glucocorticoid drug with potent anti-inflammatory and immunosuppressant effects. It is widely believed that dexamethasone improves the quality and duration of peripheral nerve blocks over local anesthetics alone. This is thought to be mediated by attenuating the release of inflammatory mediators, reducing ectopic neuronal discharge, and inhibiting potassium channel-mediated discharge of nociceptive C-fibers [26-28].

Williams et al. [29] examined in vitro neuronal death when exposed to ropivacaine and several other perineural adjuvants either alone or in combination. They demonstrated that when doubling the concentration of dexamethasone from 66 to 133 mcg/mL, increased neuronal death was observed. The authors raised this concern but failed to mention that clonidine and buprenorphine were also present in the same mixture and that the increased neuronal death was not observed with dexamethasone and ropivacaine alone. A synergistic effect could not be ruled out, and the blame should not be attributed solely to dexamethasone. Attention was also drawn to the fact that 133 mcg/mL would be considered a subclinical dose, given that 8 mg of dexamethasone translates to a concentration of 220 to 363 mcg/mL. Authors also observed no significant cell death with 667 mcg/mL of dexamethasone alone at 24 hours nor was there additional cell death when combined with ropivacaine at 2 hours with that concentration. It therefore seems plausible that dexamethasone contributes to ropivacaine-induced cell death only in the presence of other adjuvants [29]. On the other hand, steroids may have a direct neurotoxic effect on the peripheral nerve tissue only when the drugs are injected intrafascicularly into the nerve bundle [30]. Our study shows, when applied intraneurally (intrafascicular and extrafascicular), lidocaine with dexamethasone produced neurologic sequelae
that lasted more than 24 hours, and were still evident at the end of experiment on day 3. Pathohistological changes were also evident in the intraneural group of lidocaine in combination with dexamethasone. We found damages at the level of nerve fibers, Schwann’s cells, intrafascicular blood vessels, interstitium, perineurium and epineurium in all nerve samples. Those changes were present in the neighboring proximal and distal regions from the application site, but they were of weaker intensity.

Mackinnon et al. [31] studied effect of different steroid agents on nerve fibers. In comparing with other used steroids (hydrocortisone and triamcinolone), dexamethasone caused minor axonal and myelin sheath changes within nerve fibers, observed under electronic microscope. Injections of steroid agents, including dexamethasone, in extraneural epineural tissue caused no nerve damage. Dexamethasone is presumed to have peripheral nerve activity, through inhibition of C-fiber transmission, although causing minimal direct peripheral nerve damage compared with hydrocortisone or triamcinolone [31] in clinically relevant doses. Preservatives added to steroids such as benzyl alcohol and propylene glycol have known neurolytic effects, so it is important to avoid steroid adjuvants with any preservatives. Therefore, the US Food and drug administration did not approve the use of local dexamethasone as an adjuvant to local anesthetics. However, the neurotoxicity of dexamethasone has been [32,33] discussed in the pain text books and it was found that 4–12 mg of dexamethasone through epidural, perineural and intravenous routes has no neurotoxic effects. Furthermore many studies [34] have not found long term effects on the function of peripheral nerves by using locally applied corticosteroids.

The neurotoxic effects of dexamethasone manifesting by inhibition of synaptic reactivity of the studied neurons may be partially mediated by activation of NMDA glutamate receptors. It was found that glucocorticoids inhibit glucose uptake by both neurons and glial cells that results in the decreasing of energy production in these cells. Deficit of energy causes a decrease of activity of transporters, including synaptic neuronal and glial glutamate transporters [35,36].

The mechanistic understanding regarding dexamethasone action on the normal non-inflamed peripheral nerve is quite limited. One experimental study using electrically stimulated A-fibers and in C-fibers of the rat plantar nerve observes a direct membrane action with a suppression of the transmission in thin unmymelinated C-fibers but not in myelinated A-β fibers [37]. In contrast with the results of our research, study of Ke An et al., shows that perineural, but not systemic administration of dexamethasone, when added to clinical concentration of bupivacaine not only prolong the duration of sensory and motor blockade of sciatic nerve, but also prevent the bupivacaine-induced reversible neurotoxicity and short-term rebound hyperalgesia after the resolution of block and that the protective effect of dexamethasone is likely Schwann cell related. Neurotoxicity of plain bupivacaine identified via histomorphology and S-100 expression patterns was no longer significant with the co-administration of preservative-free dexamethasone perineurally. It is possible that perineural dexamethasone prevent the transient neurotoxicity of bupivacaine and guard against demyelination and Schwann cell degeneration, and therefore demonstrates anti-nociceptive and anti-neurotoxic effects in mouse sciatic nerve block model [38]. The previous large use of epidural steroids including dexamethasone to treat radiculopathy pain may support the absence of local nerve toxicity [39]. On the other hand, in an experimental study using isolated sensory neuron, results suggest that attention should be directed towards exploring the time-dependent and concentration-dependent basis for neurotoxicity associated with dexamethasone combined with ropivacaine [29]. In addition to this issue of the potential neurological toxicity of dexamethasone itself, we have to remember that ‘off label’ use of analgesic drugs in regional anesthesia can also expose to exicipient neurotoxic properties [40].

Dexamethasone’s ability to augment the analgesia offered by regional single-shot nerve blocks and thus improve the management of acute pain in the perioperative period is supported by all of the literature currently available. But, dexamethasone’s proven ability to generate neural damage in animal models, hyperglycemia and even sepsis with repeated administration should not be discounted [41-43]. The results of this study would suggest that dexamethasone do, indeed, have neurotoxic effect. As noted in previous studies [44,45], the site of injection is critical. The agent must be delivered directly into the nerve fascicle in order to exert any neurotoxic effect. The ideal adjuvant to peripheral nerve blockade should increase block duration and/or decrease local anesthetic dose. Perineural dexamethasone remains an off-label use and should be used cautiously only in select patients who may benefit from extended duration analgesia and in whom continuous catheter techniques are inappropriate or contraindicated. IV administration is preferable if it is truly equivalent to perineural administration for analgesic outcomes and if there is neurotoxicity. Additional studies need to be conducted to definitively answer the questions of administrative route, neurotoxic dose, and clinical benefit.
CONCLUSION

Dexamethasone has potential as an additive in regional analgesia for its ability to prolong the duration of action of analgesia afforded by 'single-shot' peripheral and neuraxial blocks. The routes of administration and dosing ranges are currently controversial but a trend to low dosing and systemic use appear to reduce the potential for complications. Results of our study showed, that when applied intraneurally dexamethasone in combination with lidocaine caused nerve fiber damage. However, future studies are required to elucidate the most effective route and optimum dosing range for dexamethasone's use in this field.

CONFLICT OF INTEREST

Authors declare no conflict of interest.

REFERENCES


