Intrafascicular application of Lidocaine 2% or 0.9% NaCl into median nerve of the rat

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ABSTRACT

Objective: Intraneural (intrafascicular) injection of various solutions can result in a mechanical injury to the fascicle(s). Additional injury can be expected when injectate has neurotoxic properties. In this study we examined the neurologic consequences of intraneurally injected lidocaine 2% and 0.9% NaCl. We postulated that intraneural injection of lidocaine 2% results in greater and longer-acting neurologic deficit in rats compared to intraneural injection of 0.9% NaCl.

Methods: The study was conducted in accordance with the principles of laboratory animal care and was approved by the Laboratory Animal Care and Use Committee. Twenty four adult Wistar rats (300 g), both sexes, were studied. After induction of general anesthesia (ether), the median nerve was exposed bilaterally. Under direct vision, a 27-gauge needle was placed either perineurally (n=24) or intraneurally (n=24), and 3 mL of preservative-free lidocaine 2% or 0.9% was injected using an automated infusion pump (3ml/min). Injection pressure data were acquired using an in-line manometer coupled to a computer via an analog-to-digital conversion board. After injection, the rats were awakened and subjected to serial neurologic examinations. Neurologic examination protocol was followed to determine grip strength and toe pinch reaction. Day 7 of the experiment, the animals were sacrificed and the neural tissue histologically examined.

Results: Over the week following the procedure, all animals in both injection protocols (intraneural and perineural) initially lost and subsequently regained grip strength and paw pinch withdrawal reflex. Animals in the perineural group fully recovered grip strength and toe pinch score within 24-hours of surgery; the saline group showed more rapid recovery. In contrast, neither the lidocaine 2% or 0.9% saline intraneural group fully recovered a grip strength or toe pinch until the 7th or 4th day of recovery, respectively. There were no differences in the extent of neurologic impairment or speed of recovery between the saline and lidocaine 2% groups. Post-hoc comparisons of the four injection group by treatment condition effect at each testing interval post-surgery showed superior recovery in the perineural injected preparations at all intervals tested after hour 4 (p < 0.001). The average peak pressure for the intraneural injection group was 80.96 ± 20.94 kPa versus 21.63 ± 5.38 kPa for the perineural injection group (p < 0.0001). Histologic features of the injured tissues ranged from perineural ablation, cellular infiltration to destruction of neural architecture and axonal degeneration in intraneural preparations. No differences in this regard were found between lidocaine 2% and saline 0.9% groups.

Conclusions: Intraneural injection of lidocaine 2% or 0.9% NaCl result in an indistinguishable neurologic deficit that is similar both in extent and duration. Intraneural injection is associated with significantly higher injection pressure as compared to the perineural injection. These results suggest that the main mechanism of neurologic injury resulting from an intraneural injection of lidocaine 2% may be a mechanical, injury to the fascicle(s), rather than a direct neurotoxicity.

Keywords: intrafascicular injection, high injection pressure, local anesthetic, neurologic injury, median nerve.

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INTRODUCTION

Nerve injury after peripheral nerve block (PNB) is a rare but serious complication of regional anesthesia. Unintended injection of local anesthetics directly into nerve (intrafascicular injection) has long been recognized as a possible cause of nerve injury [1]. There are many possible ways in which nerves can be damaged. Events such as direct injury by needles, instruments, suturing, or the injection of neurotoxic material, or even thermal insults from diathermy, can cause nerve damage. The relationship between the agent and its effect is usually unclear. The interdependence between mechanical and ischemic factors is well recognised, but which is the more important factor in individual cases remains controversial [2]. Peripheral nerve injury after intrafascicular injection may occur with various
therapeutic and other agents. Damage may be minimal or may result in severe axonal and myelin degeneration, depending on the agent injected and dose of the drug [1]. Studies in animals have suggested that intraneural application of local anesthetics may cause mechanical injury and pressure ischemia on nerve fascicles [3,4]. Additional injury can be expected when injectate has neurotoxic properties. Neurotoxicity after the use of local anesthetics (LAs) for peripheral nerve block has been recognized for decades. Peripheral nerve block is commonly performed to provide surgical anesthesia and postoperative analgesia. Loss of sensation, motor function, pain and causalgia have been reported as a result of intraneural injection of LAs. Some studies have claimed that the inadvertent intraneural injection of LAs dose not always result in lasting neurological injury or functional impairment [5,6]. Our hypothesis is that an intrafascicular injection of lidocaine 2% will result in greater and longer acting neurologic deficit in rats compared to intrafascicular injection of 0.9% NaCl.

MATERIALS AND METHODS

Animals

The study was conducted in accordance with the Principles of Laboratory Animal Care [7] and was approved by the Laboratory Animal Care and Use Committee of the Medical and Veterinary Schools of the University of Sarajevo. Twenty-four adult Wistar rats (300 g) and of either sex were used in the study. Animals were housed in central animal care facility and given rat chow and water ad libitum.

Experimental design

On the day of experiment, general anesthesia was induced using ether. Thereafter, by using an aseptic technique for survival surgery, both medianus nerves were exposed bilaterally. Under direct vision, a 27-gauge, long-bevel needle (LifeTech, PB-25SCS) was placed either perineurally on one side (n=24) or intraneurally on the contralateral side (n=24) in a random order. For perineural injections needle was placed within the epineural tissue but outside the perineurium, while for intraneural injections the needle was placed intraneurally inside the perineurium. The needle was mechanically stabilized without suturing, and 3 mL of preservative free 2% lidocaine (Bosnalijek, Sarajevo) or 3mL of NaCl (0.9%) was injected. An automated injection pump (PHD 2000 Harvard Apparatus, Holliston, MA) administered the injections at a speed of 3mL/min. Injection pressure data were acquired by using an in-line manometer (PG5000; PSI-Tronics Technologies Inc, Tulare, CA) coupled to a computer via an analog-digital conversion board (DAQ 6023; National Instruments, Austin, TX). The manometer was placed proximal to the needle and in line with nondistensible high durometer polyvinyl chloride injection tubing (84° arterial pressure tubing; Abbot Critical Care Systems, Abbot Laboratories, North Chicago, IL). Pressure data were analyzed by using a data analysis software package (BioBench version 1.2; National Instruments, Austin, TX). In this study we used BioBench program in order to register and analyze the values of pressures during intraneural and perineural application. After injection, the incision was closed and the animals were allowed to awake, the methodic neurological examination was performed in certain time intervals: every hour on first 8 h after awakening, and one daily in the next 7 days. Neurological examination was conducted by Thalhammer’s neurological examination [8], and included assessment for motor function and nociception by following criteria:

Motor function was evaluated by measuring Grip strength test by Grip strength system (San Diego Instruments, Ohio, USA), used for the evaluation of neuromuscular function of laboratory animals by recording the maximum amount of power that animal is performed by catching especially constructed grid on which is placed its front limbs. It consist of an acrylic platform easy to disassemble and clean, digital power meter and the sensory mechanism in the form of a grid (Figure 1). Animals are kept for nuchal translucency and base of the tail and down the grip strength platform until we are sure that its front limbs grabbed grid. Then the animal is brought back to the tail in a horizontal plane. After rat drop grid device automatically record the maximum tractive power on grid and is displayed on the display. The duration of the motor blockade was defined as the time required for recovery to 25% of motor deficit.

Figure 1. Grip strenght system

Nociception was evaluated by observing the withdrawal of the limb (toe pinch reaction) in response to a noxious stimulation as:

4 – normal withdrawal reaction, brisk withdrawal of the paw, vocalization, bites the forceps;
3 – slower withdrawal reaction, weaker withdrawal of the pinched extremity, vocalization, no attempts to bite the forceps;
2 – slow withdrawal reaction, no vocalization, no attempts to bite 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 lasing of block is defined as time which passes until the response returns to score 3 (75% of normal).

On day 7 of the experiment, the rats were killed by using an overdose of sodium pentobarbital and potassium chloride, and 3-cm-long specimen of medianus nerve, containing the injection site, was excised from each side. The tissues were fixed in formalin, embedded in paraffin, and stained with hematoxylin and eosin for histologic examination. Qualitative histological analysis of the samples was performed by pathologist blinded to the study groups.

Statistical analysis
Forty-eight medianus nerves (24 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 α=0.05 [9]. Statistical analyses were performed by using SPSS program, version 11.5. Maximum pressure values during intraneural and perineural injections were compared by using paired t-test. Rates of neurologic injuries were compared between intraneural and perineural injections by using McNemar’s test for paired proportions. A P value < 0.05 was considered to be significant.

RESULTS
Pressure Data
All injection were characterized by increase of pressure in the beginning of the application, resulting in maximum pressure, which was then followed by significantly lower pressure during the remaining part of the application.

The average value of maximum pressure achieved in peak effect for all intraneural injections was 80.96 ± 20.94 kPa (the average value ± standard deviation), in comparison to 21.63 ± 5.58 kPa for perineural group (P < 0.0001).

Neurologic Data
Over the week following the procedure, all animals in both injection protocols initially lost and subsequently regained grip strength and paw pinch withdrawal reflex, except perineural injection of 0.9% saline, who did not resulted in any neurological impairment. Mixed model analysis of variance for repeated measures showed that differences in injection procedure were larger than differences in treatment condition on the temporal course of change in these functional measures. For grip strength, the pattern of function loss and recovery differed by injection group (group by time interaction F_{[11, 211]} = 9.27, p < 0.0001) but not by treatment condition (condition by time interaction F_{[11, 211]} = 1.17, p < 0.31). Specifically, perineural group preparations recovered grip strength within 24-hours of surgery whereas the intraneural group preparations did not recover a grip strength value of 4 until the 7th day of recovery. By contrast, the lidocaine and saline treatment conditions did not significantly differ from each other after the 8-hour measurement. The overall injection group by treatment condition interaction was statistically significant (F_{[1, 58]} = 4.08, p < 0.05). Examination of the interaction effect of injection procedure and treatment condition over the recovery period showed that the perineural-saline and the perineural-lidocaine preparations recovered half grip strength by 8-hours, whereas the intraneural-saline and intraneural-lidocaine preparations did not achieve this level until day 4. Post-hoc comparisons of injection group by treatment condition effects confirmed this trend in the course of recovery for behavioral testing at each post-surgical testing time after hour 4 (p < 0.0001).

Overall results for paw pinch reflex mimicked those for grip strength: significant interaction effect of injection group by time (F_{[11, 211]} = 7.11, p < 0.05); significant interaction effect of injection group by treatment condition (F_{[1, 48]} = 4.56, p < 0.04); but, unlike grip strength, a significant treatment condition by time interaction (F_{[11, 211]} = 2.00, p < 0.03). Briefly, toe pinch recovered from 0 to 3 by hour 8 in the perineural group but did not reach this level until hour 96 in the intraneural group. The lidocaine and saline groups both achieved toe pinch scores over 3 by the second post-surgical day, although the saline group showed slightly more rapid recovery during the first 8 hours after injection (Figure 2). Like the findings for grip strength, both the perineural saline and lidocaine treated preparations achieved toe pinch scores above 3 by hour 8 where as neither intraneural injected saline or lidocaine treated preparation achieved this level of recovery prior to hour 96. Post-hoc comparisons of the four injection group by treatment condition effect at each testing interval post-surgery showed superior recovery in the perineural injected preparations at all intervals tested after hour 4 (p < 0.0001).

Pathohistologic Data
Macroscopic observation of the median nerve in intraneural group showed a fusiform swelling of the all nerves at the site of injection, extending approximatel 1 to 2 cm in the proximal-distal direction with partially herniation at the site of application. Perineural injections resulted in the spread of the injected solution within epineurium, and its leakage into the sur-
rounding tissue. Histological examination of nerves in intraneural group revealed ablation of perineurium, its loss of layers of lamellas. At the site of injection of the needle perineurium was significantly disintegrated or ruptured. It was observed intraneural hypercellularity. Nerve fibers showed varying degrees of damage, from enlargement of voluminosity, the disintegration of the myelin sheath, hyper-acidophilia of the cytoplasm, to the disintegration of the nerve fibers. Axons showed evidence of severe nerve injury with widespread degeneration of axons and myelin sheath sequestration (Figure 3, Figure 4). No differences in this regard were found between lidocaine 2% and 0.9% saline groups.

Figure 2. Paw grip recovery

Epineurium is edematous with infiltration of inflammatory elements and hyperaemic blood vessels. Perineurium shows disintegration and division of lamellas, with the loss of normal compactness with a distinct subperineurally edema which is manifested partially in a deeper area of fasciculus. Nerve fibers shows a different degree of damage, change in a position of the axon within the fibers, lysis of axons, uneven diameter of the fibers.

Figure 3. Intraneural application of 0.2% lidocaine with high injection pressure (HE, X 40)

Ep – epineurium; Pe – perineurium; Nf – nerve fibers; Ic – inflammatory cells; Ed – edema

Figure 4. Intraneural application of 0.9% saline with high injection pressure (HE, X 40)

Ep – epineurium; Nf – nerve fibers; Noticeable is invagination of connective tissue in epineurium and field of damage inside the epineurium and perineurium, which continues on the fasciculus and nerve fibers, which probably corresponds to where the penetration of the needle happened (marked with arrow).
DISCUSSION

Peripheral and neuraxial regional anesthesia techniques are widely used worldwide [10]. These methods primarily rely on the injection of local anesthetics (LA) to reversibly block neuronal voltage-gated sodium channels (VGSC) and to thereby reversibly interrupt nerve impulse propagation [11]. Overall, this leads to a reduction in perioperative pain and systemic effects of surgical stress after certain types of surgery [10]. Besides of the nervous system, LA have a positive influence on the inflammatory response and the hemostatic system. In particular, G-protein coupled receptors of the G_{q/11} subfamily are involved in anti-inflammatory effects of LA, and the intravenous administration of lidocaine has been shown to duplicate a substantial share of the effects of regional anesthesia after visceral surgery [10,12]. Peripheral nerve block with LA is a common practice in providing pain control for a wide range of surgical procedures and pain syndromes [13].

Inadvertent intrafascicular injection of an LA can generate a variety of nerve injuries, some of which may result in long-term disability [13]. Nerve injury from peripheral nerve blockade is well described; however, case reports of incidence, duration and extent of sequelae are varied. Nerve injection injury is considered to be multifactorial in nature [6]. The clinical phenomenon of local anaesthetic toxicity has been known for more than a hundred years [14]. Events such as direct injury by needles, instruments, suturing, or the injection of neurotoxic material, or even thermal insults from diathermy, can cause nerve damage. The relationship between the agent and its effects is usually unclear. Less obvious, but more frequent adverse events, involve mechanical factors such as compression, stretch, angulation, percussion or transection [2, 6, 15].

Results of our study showed, that all intraneural injection, lidocaine 2% or saline 0.9%, resulted in motor and sensory block that last through out day 7 of our experiment, which clearly showed that nerve damage is caused by mechanical factors rather then the type of injected solution. On contrary, motor and sensory blocks caused by perineural injection were recovered within 24 h after injection in all groups. In other words, our results show that the place of application is crucial factor in determination the grade of nerve injury. In general, most drugs caused nerve injury when injected intrafascicularly, and in contrast, extrafascicular injections produced little to no damage. A few exceptions, dexamethasone, botulinum toxin, and bovine collagen, demonstrated little to no axonal damage after intrafascicular injection [6, 16]. Severally recently published reports document the inadvertent intraneural placement of the needle and subsequent injection of LA without clinically apparent neurological injury.

Contrary to our and many others results Iohom et al. [17], in 2005. applied intraneurally Ropivacaine into sciatic nerve in rat and concluded that intraneural injection of Ropivacaine has no noxious effect on nerve motor function. In contrast to our results, those authors did not define well exact position of the tip of the needle. In our study we used injection pressure for the determination where tip of the needle of instrument is located (intrafascicular or extrafascicular). Our data suggest that neurological injury does not always develop, even after intraneural injection, because block may be results of the intraneural but extrafascicular deposition of local anesthetic [1, 18].

Perineural application of LA significantly reduces neurotoxic potential, meaning that it carries very small risk of nerve damage. The reason for this is probably the fact that in normal circumstances applied amount of LA equalizes pressure with surrounding tissue. In that moment the diffusion into surrounding tissue occurs, the interstitial liquid rapidly dilutes LA and its concentration further decreases by system absorption [19]. As in previous studies, in our study as well all perineural injections, independet to the kind of applied solution did not caused permanent motor and sensory block.

In addition to toxicity of the medication used, the high intraneurial pressures achived during accidental injection of drug into nerve have been shown to be damaging. In our study all intraneural injection resulted in significantly larger maximum pressure achived in peak effect, in contrast to perineural injections (P<0.0001).

Hadzic et al. [1] found that when using the same injectate volumes, intrafascicular injections resulted in higher pressured and were associated with lasting motor deficits and histological damage. This results well corresponds with the results of Kapur et al. [20], who found that intrafascicular application of lidocaine in sciatic nerves in pigs, resulted in higher application pressure and intensive histologically changes.

The neurological consequences of an intraneural needle insertion and injection of LA depend on many factors such as design of the needle tip, injection force and the chemical structure and concentration of the injectate [21]. Nerves may be unduly susceptible to trauma as a result of a pre-existing generalised peripheral neuropathy or a local compression neuropathy [22,23]. However, the anatomic specifies of a given nerve and how its organization can protect it against external injury may be the most significant factor that determines the likelihood of injury [21].

The most important anatomic factor that determines the vulnerability of a given nerve to an intraneural needle insertion or injection of LA is probably ration of connective (epineural) tissue to axonal tissue (fascicle) and the size of nerve fascicle or fascicular bundles. The lesser the quantity of connective tissue and the larger...
the fascicles, the higher the chances are that an advancing needle can enter the fascicle [21].

The neurotoxic effect of the local anaesthetics are probably mediated by mechanism other than Na+ channel blockade. The proposed models of neuronal injury include depletion of ATP, mitochondrial injury and prolonged elevation of a cytosol Ca++ [24].

Peripheral nerve injury after intrafascicular injection may occur with various therapeutic and other agents [25,26]. Damage may be minimal or may result in severe axonal and myelin degeneration, depending on the agent injected and dose of the drug used [27,28,29].

Our data are in agreement with findings of earlier studies showing that intraneural injection increases the risk of nerve injury. In our study, histologic examination of nerves with intraneural injection of lidocaine 2% or 0.9% saline, revealed division of lamellas in perineurium with significant disintegration at the place of puncture and the loss of demarcation toward the surrounding perifascicular connective tissue and closest nerve fibers. Some of the axons of these fibers were dislocated and hyperacidophile, and hypercellularity can be noticed, with no differences in this regard were found between lidocaine 2% and 0.9% saline groups. Other authors mentioned similar histological changes during intraneural application as well, but not in all cases because they did not distinguish between intra- and extraneural application [1, 16, 30, 31]. In our study high injection pressure was reliable indicator of intrafascicular placement of needle, and all intrafascicular injection resulted in significant histological changes and nerve damage.

While some authors consider that for the emergence of nerve defect multi-factorial impact is needed (mechanical trauma and toxic effect of LA), others showed that the main cause of nerve injury during application of intrafascicular injection of mechanical trauma [20]. Pressure of the intraneurally injected LA may also add to the potential for ischemia by compression of capillaries and venules. Direct chemical toxicity from the LA itself, or additives such as epinephrine, is also enhanced in the presence of a mechanical trauma to the nerve fascicles and disruption of the perineurium [32]. The mechanism of injury with intrafascicular injection includes a combination of direct mechanical injury, changes in the permeability of the blood – nerve barrier, associated oedema, pressure ischemia, epinephrine – mediated vasoconstriction and increased endoneural fluid pressure, all of which contribute to the nerve injury [27, 33, 34]. Many reported cases in literature cite combinations of several factors as responsible for nerve injury. The alternate causes of nerve injury other than the neurotoxicity of local anesthetics are mechanical. In addition, there are surgical and patient related factors. The application of one or more stressors on a dysfunctional but clinically normal nerve can result in new neurologic symptoms. Upton and McComas [35] have called this phenomenon the “double crush” syndrome. Therefore, it may not always be possible to determine a single cause to perioperative nerve injuries.

**Conclusions**

Intraneural injection of lidocaine 2% and 0.9% saline both resulted in: an indistinguishable neurologic deficit similar in extent and duration, both were associated with significantly higher injection pressure, and both resulted in severe histological damage of the nerve fiber as compared to the perineural injections. Combination of intraneural needle placement and high injection pressure leads to severe fascicular injury and persistent neurologic deficits, independent to the kind of applied solution, which suggest that the main mechanism of nerve damage and neurologic impairment may be mechanical injury to the fascicle, rather than a direct neurotoxicity.

**Conflict of interest**

The authors declare no conflict of interest.
HASANBEGOVIĆ ET AL: INTRAFASCICULAR APPLICATION OF LIDOCAINE AND NACL INTO MEDIAN NERVE OF THE RAT

REFERENCES


