



Effects of intraneural and perineural injection and concentration of Ropivacaine on nerve injury during peripheral nerve block in Wistar rats

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ABSTRACT

Introduction: Injury during peripheral nerve blocks is relatively uncommon, but potentially devastating complication. Recent studies emphasized that location of needle insertion in relationship to the fascicles may be the predominant factor that determines the risk for neurologic complications. However, it is well-established that concentration of local anesthetic is also associated with the risk for injury. In this study, we examined the effect of location of injection and concentration of Ropivacaine on risk for neurologic complications. Our hypothesis is that location of the injection is more prognostic for occurrence of nerve injury than the concentration of Ropivacaine.

Methods: In experimental design of the study fifty Wistar rats were used and sciatic nerves were randomized to receive: Ropivacaine or 0.9% NaCl, either intraneurally or perineurally. Pressure data during application was acquired by using a manometer and was analyzed using software package BioBench. Neurologic examination was performed thought the following seven days, there after the rats were sacrificed while sciatic nerves were extracted for histological examination.

Results: Independently of tested solution intraneural injections in most of cases resulted with high injection pressure, followed by obvious neurologic deficit and microscopic destruction of peripheral nerves. Also, low injection pressure, applied either in perineural or intraneural extrafascicular area, resulted with transitory neurologic deficit and without destruction of the nerve normal histological structure.

Conclusions: The main mechanism which leads to neurologic injury combined with peripheral nerve blockade is intrafascicular injection. Higher concentrations of Ropivacaine during intrafascicular applications magnify nerve injury.

Keywords: Ropivacaine neurotoxicity, intraneural injection, perineural injection.

INTRODUCTION

Injury during application of peripheral nerve blocks (PNB) is relatively uncommon, but potentially devastating, complication of regional anaesthesia. Possible mechanisms of neurologic injury may be related to mechanical needle injury, injection force, vascular

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injury, or neurotoxicity of local anesthetics and their additives (e.g., vasoconstrictors).

Recent studies emphasized that location of needle insertion and injection of local anesthetic (LA) in relationship to the fascicles may be the predominant factor that determines the risk for neurologic complications (1,2). However, it is well-established that concentration of the injected solutions in the vicinity of the nerves and duration of exposure to LA are also associated with the risk for injury (3-5). In this study, we examined the effect of location of injection (intra-neural *vs.* perineural) and concentration of Ropivacaine on risk for neurologic complications in Wistar rats.

We hypothesized that location of the injection during application of peripheral nerve blocks has higher prognostic value in occurrence of nerve injury over concentration of injected Ropivacaine.

METHODS

After animal care Ethics committee approval of the University of Sarajevo, 50 adult Wistar rats were used in experimental designed type of the study. The animals were anesthetized with an intraperitoneal injection of pentobarbital 50 mg/kg. The sciatic nerve was surgically exposed bilaterally to insert a 27 G needle (Terumo Europe NV, Leuven, Belgium) intraneurally on one side and perineurally on the contralateral side, laterality determined randomly (by the method of sealed envelopes). 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 selection of concentration of 2 ml of 0.2%, 0.5%, 0.75%, and 1% Ropivacaine or 0.9% NaCl was randomized (using a computer-generated sequence). An automated injection pump (PHD 2000 Harvard Apparatus, Holliston, MA) administered the injections at a speed of 5 ml/min. Injection pressure was continuously recorded using an in-line digital manometer (BioBench). Increased injection pressure was used to distinguish intrafascicular from extrafascicular intra-neural injections.

After injection, animals were allowed to wake up from anaesthesia and were given a series of neurologic examinations according to Thalhammer (6).

Neurologic examinations were performed hourly for the next 6 hours and daily for the next 7 days, and included assessment of proprioception, motor function and nociception by the following criteria:

- 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 was evaluated). The functional deficit was graded as: 0 - normal; 1 - slightly impaired; 2 - severely impaired; 3 - absent.
- Motor function was evaluated by measuring the extensor postural thrust: the rat was held upright with the hind limb extended so that the body's weight was supported by the distal metatarsus and toes and the extensor postural thrust could be measured as the force applied to the digital balance, the force that resists 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 a percentage of the control force.
- Nociception was evaluated by observing the withdrawal of the limb in response to a 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 7 days after injection of the test solutions, and specimens of the sciatic nerve on block with neighboring tissues were removed. The samples were fixed in formalin and paraffin followed by microtomial sections and stained with hematoxylin and eosin methods. Qualitative

histological analysis of the samples was performed by pathologist blinded to the study groups.

Statistical analysis

A study sample size of 100 sciatic nerves (50 rats) were required for the 80% power and a 5% type I error rate for a two-tailed T-test designed to detect a 1.5 SD difference in peak injection pressure in two groups defined as perineural vs. intraneural injections. Rates of neurologic and histological injuries were compared between intraneural and perineural injection by using McNemar's test for paired proportions. Fisher's exact test was used to compare injury rates during the intraneural injection, based on injected solution. Statistical analysis was performed by using SPSS and a p value of <0.05 was considered to be significant.

RESULTS

Injection pressures

All experiments were completed as planned. All perineural injections resulted with the low pressure (≤ 24.5 kPa), while the majority of intraneural injections resulted with the high pressure (≥ 109.8 kPa). Only two intraneural injections resulted in lower injection pressures which are indicated as intraneural extrafascicular injections.

During intraneural applications the maximum pressure was 187.3 kPa, while the minimum pressure was 26.4 kPa, achieved in peak effect. Maximum pressure reached in all perineural applications was 24.5 kPa and minimum was 14.6 kPa, also achieved in peak effect. The average value of maximum pressure achieved in peak effect for intraneural injection was 138.1 ± 30.9 kPa (mean value \pm standard deviation), in comparison to 16.9 ± 1.9 kPa for perineural injection ($p \leq 0.05$). The difference between average values of intra and perineural injections (with 95% confidence interval) was statistically significant ($t=3.14$; $DF=6$; $p=0.02$).

Neurologic outcome

After recovery from general anesthesia, sensory-motor sciatic blockade was evident in rats that received Ropivacaine in each concentration but not in rats received saline.

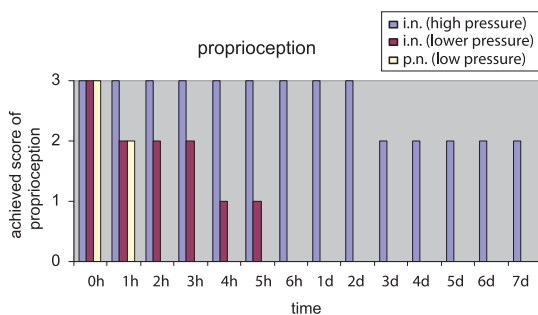


FIGURE 1. Proprioception of rat's hind limb after injection application; i.n. – intraneural; p.n. – perineural.

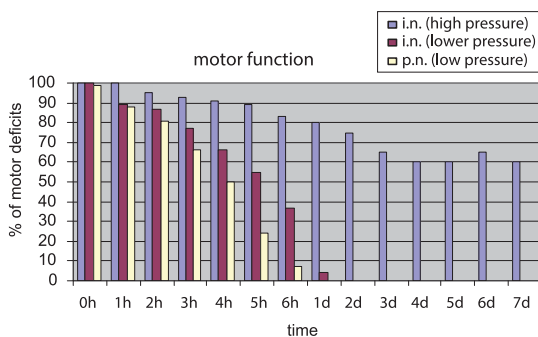


FIGURE 2. Motor function of rat's hind limb after injection application; i.n. – intraneural; p.n. – perineural.

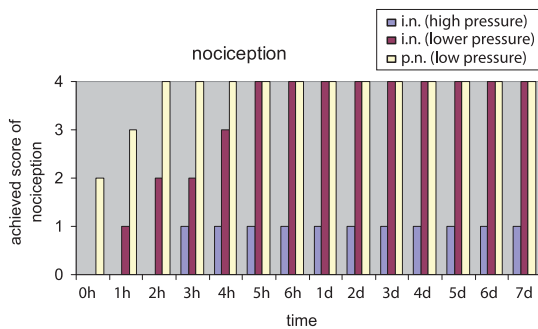


FIGURE 3. Nociception of rat's hind limb after injection application. i.n. – intraneural; p.n. – perineural.

Following neurological exam, it has been found that all intraneural injections which were associated with high application pressure (independent of the tested solution and concentration) resulted with deficits which lasted more than 24 hours, and neurological deficits were evident also at the end of experiment,

after 7 days, which clearly shows that intraneural intrafascicular injection caused the nerve damage.

On the contrary, all injections associated with low injection pressure, whether they were intraneural or

perineural didn't result with neurological sequels at the end of the experiment ($p < 0.05$). Furthermore, in most cases neurological deficit has withdrawn within first 24 hours of experiment, (Figure 1-3).

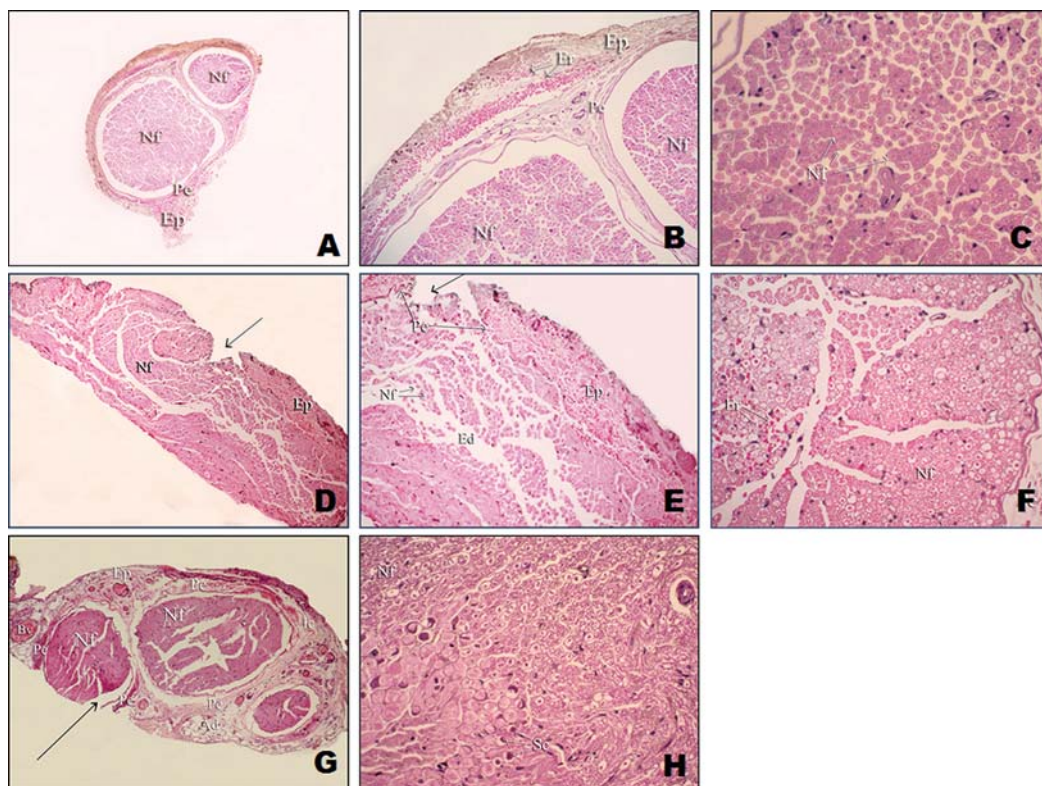


FIGURE 4. (A) Perineural application of 0.75% Ropivacaine with low injection pressure (HE, X10). Cross-section of rat's sciatic nerve composed of two nerve fasciculus. Connective tissue of nerve and nerve fibers preserved structures. (B) Perineural application of 0.75% Ropivacaine with low injection pressure (HE, X100). Epineurium infused with erythrocytes. Perineurium lamellas preserved, as well as structure of nerve fibres intrafascicular. (C) Perineural application of 0.75% Ropivacaine with low injection pressure (HE, X400). No deviation from the normal histological structure of nerve fibers visible intrafascicular. (D) Intraneural application of 0.2% Ropivacaine with high injection pressure (HE, X40). Noticeable invaginations of epineural connective tissue (indicated by arrow), with loss of structural space intrafascicular. Perineurium is shown as division of lamellas with its significant disintegration, while nerve fibers evidence of nerve injury. (E) Intraneural application of 0.2% Ropivacaine with high injection pressure (HE, X100). Visible damage of epineurium, perineurium, which continues to the fasciculus and nerve fibers, which probably corresponds to place of needle penetration (marked arrow). Diffuse damage of nerve fibers. (F) Intraneural application of 0.2% Ropivacaine with high injection pressure (HE, X250). Nerve fibers are disarranged in the space and of increased volume. Most of the axons of those fibers are dislocated and hyperacidophile. Advanced axolysis and myelin disintegration is noticed. Some of the erythrocytes are located extravasally. (G) Intraneural application of 1% Ropivacaine with high injection pressure (HE, X40). Place the cursor shows a marked rupture of perineurium. Degenerative changes through entire fasciculus are noted. Groups of adipocytes with hyperemic blood vessels are evident. (H) Intraneural application of 1% Ropivacaine with high injection pressure (HE, X250). Diffuse axonal swelling and an advanced axolysis up to degree of complete disintegration was apparent. No normal axons are seen. Schwann's cells are enlarged with hyperchromatic nuclei. Ep-epineurium; Pe-perineurium; Nf-nerve fibres; Er- erythrocytes; Ed-edema; Ad- adipocytes; Ic- inflammatory cells; Bv-blood vessels; Sc- Schwann's cells.

Histopathological examination

Histological examination revealed normal sciatic nerve structure in all low pressure injections (50 perineural and 2 intraneural extrafascicular). Perineural and extrafascicular injections (independent of the tested solution and concentration) didn't result in any significant nerve injury as assessed by light microscopy (Figure 4 A-C).

By contrast, pathological changes which varied from occasional nerve fiber injury at the site of injection to severe axonal and myelin degeneration were observed following intrafascicular injections. In addition marked cellular infiltration, subperineurial edema and diffuse axonal swelling was apparent in most of intrafascicular injections, (Figure 4 D-H). Pathological conditions in the periphery of the fascicle were more prominent than in central zone. With higher concentration of Ropivacaine injected intrafascicular pathological findings were more marked, with evidence of wide-spread axonal and myelin degeneration of the entire fascicle. The high injection pressure group had a significantly greater rate of injury (98%) as compared with the low injection pressure group. Using higher concentration of Ropivacaine during intrafascicular injections the degree of nerve injury was increased (0%; Fisher exact test $p=0.03$).

DISCUSSION

Peripheral nerve blockade with local anesthetics is common practice in providing pain control for wide range of surgical procedures and pain syndromes. Inadvertent intrafascicular injection of a local anesthetic can generate a variety of nerve injuries, some of which may result in long-term disability (7). Ropivacaine in any concentration used in this study produced some degree of damage to the nerve when injected intrafascicularly, as evidenced by disintegration and demyelization of nerve fibers.

It is well known that all local anesthetics are potentially neurotoxic if they have been used in higher concentrations than prescribed or if they act on nerve through prolonged time period (8). However, the previous experience shows that perineural application of local anesthetic significantly reduces neurotoxic potential, meaning that it carries very small risk of nerve damage. The reason for this is prob-

ably the fact that in normal circumstances applied amount of local anesthetic equalizes pressure with surrounding tissue. In that moment the diffusion into surrounding tissue occurs, the interstitial liquid rapidly dilutes local anesthetic and its concentration further decreases by system absorption. As in previous studies, in our study as well all perineural injections of Ropivacaine (independent of concentrations used) have not resulted with significant damage of nerve fibers.

In contrast to perineural injections, the intraneural injections of local anesthetic may result with nerve damage (9,10). Our results correspond with results from previous studies showing that intraneural injection increases the risk of nerve damage.

While some authors consider that for the emergence of nerve defect multi-factorial impact is needed (mechanical trauma and toxic effect of local anesthetic), others showed that the main cause of nerve injury during application of intrafascicular injection is mechanical trauma, depending on the kind and dose of applied solution and on the addition of epinephrine, we can find various types of nerve damages (11). In our study most of intraneural injections, independent to the kind of applied solution and concentrations, also associated with high injection pressure (48 out of 50 injections) have resulted with persistent neurological deficit, which shows that mechanical insult caused by intrafascicular placed needle is critical in the occurrence of nerve injury. In other words, our results show that the place of application is crucial factor in determination the grade of nerve injury. Higher concentrations of Ropivacaine applied intrafascicular resulted with increased level of nerve injury.

Contrary to our and many others results Iohom and associates in 2005 applied intraneurally Ropivacaine into sciatic nerve of rat and concluded that intraneural injection of Ropivacaine has no noxious effect on nerve motor function (12). Unfortunately authors ignored that intraneural injection can be intra or extrafascicular, what would have great impact on final outcome. Also, authors analyzed only motor function, without examination of sensory function and without histological verification of those findings. Whitlock et al showed that Ropivacaine is associated with marked histological abnormality when

injected intrafascicular, while milder histological damage were seen when Ropivacaine was injected extraneurally or extrafascicular (13). The authors used finger pressure to distinguish intrafascicular and extrafascicular injections. Unfortunately, anesthesiologists often rely on subjective estimation of abnormal resistance to injection using finger pressure during the performance of peripheral nerve block, knowing that intraneural injection results with bigger resistance to needle. Hadzic and associates showed that the perception of the resistance can rather vary among the anesthesiologists, that this method is inconsistent and can be affected by different designs of needles (14).

Selander and Hadzic have demonstrated that intraneural injections into sciatic nerve of the dog in most of cases were combined with high injection pressure, while perineural injections were associated with low application pressure (15,16). In our study 48 intra neural injections were combined with injection pressure higher than 109 kPa, while neither one perineural injection was resulted with pressure higher than 24.5 kPa. Even more important, intraneural high injection pressures in our study were also associated both with neurological deficit and histological evidence of injury to nerve fascicles.

Two low pressure intraneural injections did not result in neurological consequences because the needles were not lodged intraneurally but between the fascicles instead of intrafascicular. Since peripheral nerves have natural protective mechanisms, like relative resistant membrane of perineurium, it is hard to assure intrafascicular lodged needle. In that case local anesthetic is deposited out of fascicle and such blockade lasts for hours after injection, but there is no histological evidence of nerve fibers damage.

In our study fascicular injury and neurological deficit were developed only after intraneural injection joined with high injection pressure. Study of Kapur and associated gave similar results (17). In fact, they used sciatic nerves of dogs and injected 20 intra neural injections in which only 8 resulted with intrafascicular lodged needle and were combined with high injection pressure. Remaining 12 intraneural injections were combined with lower injection pressure and there was no evidence of nerve fibers injury. Opposed to the mentioned study in our study num-

ber of intraneural extrafascicular injection was much lower (2 from 50). The reason is that sciatic nerves differ in rats and dogs. In rats nerve is composed mostly from 1 big and 1-2 small fascicles with little epineural tissue. It is not case with sciatic nerve of dogs, pigs, rabbit and humans, where nerve is mostly multifascicular or composed from more equal fascicles with extensive epineurium. This is the reason why in other species was more difficult lodging the needle intrafascicular than in our case.

Besides proven neurotoxic properties of Ropivacaine when injected intrafascicular, feature which is characteristic for all other local anesthetics, Ropivacaine applied perineurally is good choice for intraoperative and postoperative regional anaesthesia and analgesia. In general, all tested solutions caused nerve injury when injected intrafascicularly, and in contrast, extrafascicular injections produced little to no damage. This clearly showed that place of injection is crucial factor in determining nerve injury, while high concentration of LA only amplifies the level of injury.

CONCLUSIONS

Combination of intraneural intrafascicular needle placement and high injection pressures leads to severe fascicular injury and persistent neurologic deficits. The main mechanism which lead to neurologic injury combined with peripheral nerve blockade is intrafascicular injection. Higher concentration of Ropivacaine during intrafascicular applications magnify nerve injury. Ropivacaine applied in intrafascicular space is neurotoxic, similar like any other local anesthetics. Ropivacaine applied in perineural area is potent long lasting local anesthetic appropriate for intraoperative and postoperative regional anaesthesia and analgesia.

COMPETING INTERESTS

The authors declare no competing interests.

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