Original Article

# Detection of nerve structures during peripheral nerve blockade in pigs model

Ilvana Hasanbegovic, Eldan Kapur, Amela Kulenovic, Asija Gojacic[1], Amela Dzubur[2], Jasmin Musanovic[3], Esad Pepic[4]

Departments of Anatomy, [2]Public Health and Preventive Medicine, [3]Biology and Human Genetics, [4]Pathophysiology, Sarajevo University School of Medicine, [1]Clinical Pathology and Cytology, Clinical Center University of Sarajevo, Bosnia and Herzegovina

**Address for correspondence:**
Dr. Ilvana Hasanbegovic,
Department of Anatomy, Sarajevo University School of Medicine, Cekalusa 90, Bosnia and Herzegovina.
E-mail: ilvana2810@hotmail.com

## ABSTRACT

**Objective:** In recent years regional anesthesia has gained great popularity. However, like any other medical procedure, the regional anesthesia carries certain risk of unintended intraneural injection and consequential neurological complications. Studies in animals have suggested that intraneural application of local anesthetics may cause mechanical injury. Previous studies, however, have used small animal models and clinically irrelevant injection speed or equipment. In this study we used equipment and injection methods in common clinical use to study the consequences and pressure dynamics of intraneural injection. Our hypothesis is that an intraneural injection is heralded by higher injection pressure and leads to neurologic impairment in pigs. **Materials and Methods:** Ten pigs of mixed breed (21-26 kg, 4-6 months old) were studied. After general anesthesia, the sciatic nerves ($n = 20$) were exposed bilaterally. Under direct vision, a 25-gauge insulated nerve block needle was placed either intraneurally ($n = 10$) or perineurally ($n = 10$), and 4 ml of preservative-free lidocaine 2% was injected using an automated infusion pump (15 ml/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 animals were awakened and subjected to serial neurologic examinations during next 7 days. **Results:** All perineural injections resulted in injection pressures below 40 kPa. In contrast, intraneural injections resulted in significantly higher peak pressures ($P < 0.01$). In seven (70%) intraneural injections, the injections pressures were over 140 kPa (140-350 kPa). Neurologic function returned to baseline within 24 hours in all sciatic nerve receiving perineural injections. In contrast, residual neurologic impairment was present in seven sciatic nerves after intraneural injection and was associated with injection pressures >140 kPa. **Conclusion:** High injection pressure (>140 kPa) predicts intraneural injection and consequential neurologic deficit. As long as the injection pressure is low, injection into poorly compliant tissue can be avoided and neurological complication can be prevented.

Key words: Injection pressure, local anesthetics, neurologic injury

## INTRODUCTION

In recent years regional anesthesia has gained great popularity. However, like any other medical procedure, even regional anesthesia carries a certain risk of unwanted complications. The risk of neurologic deficit, although a small one, is potential complication of blockades of all periphery nerves.[1,2]

Intraneural injection of local anesthetics has long been recognized as a cause of nerve injury after peripheral nerve blocks. However, in current clinical practice, development of nerve localization and injection

Access this article online

Quick Response Code:

Website: www.archivepp.com

DOI: 10.4103/2045-080X.112984

monitoring techniques to reliable prevent intraneural injection remains elusive.

Studies in animals have suggested that intraneural application of local anesthetics may cause mechanical injury and pressure ischemia of nerve fascicles.[3,4] Previous studies, however, have used small animal models and clinically irrelevant injection speed or equipment. Consequently, the results of those studies remain of questionable relevance to clinical practice. In this study we used equipment and injection methods in common clinical use to study the consequences and pressure dynamics of intraneural injection.

Our hypothesis is that an intraneural injection is heralded by higher injection pressure and leads to neurologic impairment in pigs.

## MATERIALS AND 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, Sarajevo University School of Medicine. Ten pigs of mixed breed (21-26 kg, 4-6 months old) were studied. On the day of experiment, general endotracheal anesthesia (halothan in oxygen) was administered after premedication with diazepam (0.05 mg/kg), atropine (0.04 mg/kg SC), and ketamine (10 mg/kg IM). After general anesthesia, the sciatic nerves ($n$ = 20) were exposed bilaterally. Under direct vision, a 25-gauge insulated nerve block needle (ProBlock, LifeTech, Stafford, TX) was placed either intraneurally ($n$ = 10) or perineurally ($n$ = 10), and 4 ml of preservative-free lidocaine 2% was injected using an automated infusion pump (PHD2000; Harvard Apparatus, Holliston, MA), with the speed of 15 ml/min.

In this study the following methods have been used:

1. Measuring and analyzing of intraneural and perineural injection pressure.
2. Evaluation of neurological status of the animals.

- Injection pressure data were acquired using an in-line manometer (PG5000; PSI-Tronics Technologies Inc, Tulare, CA) coupled to a computer via an analog-to-digital conversion board (DAQ card 6023; National Instruments, Austin, TX). The data on pressures have been analyzed using the special software package Bio Bench 1.2 (National Instruments, Austin, TX) assigned for registration and processing of data obtained in various medical researches as well as for educational purposes. In this study Bio Bench program has been used in the term of registration and processing of pressure values during intraneural and perineural application, while also at the same time registering the time interval needed for application.
- After executed injection application and awakening of animals from general anesthesia the methodic neurological examination has been implemented, in certain time intervals (immediately after awakening, each two hours during the 12 hours of first day, and one time during next 7 days). Neurological examination has been conducted by modified Thalhammer's neurological examination,[5] and included assessment for the presence and severity of paresis, ataxia, and nociception by the following criteria.

### Paresis

0-absent; 1-slight paresis; 2-moderate paresis; 3-severe paresis; 4-flaccid extremity. *Ataxia:* 0-no ataxia; 1- slight ataxia; 2-modarate ataxia; 3-severe ataxia.

### Nociception

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 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.

### Statistics

Twenty sciatic nerves were required for the power of 0.80 to detect a significant difference in proportions of nerve injury between intraneural and perineural injection at $\alpha = 0.05$. Statistical analysis has been executed by using SAS (SAS for Windows, version 9; SAS Institute Inc, Cary, NC). Maximum pressure value during intraneural and perineural injection has been compared using paired $t$-test. The occurrence of neurological injuries is compared between intraneural and perineural injections using McNemar's test for paired proportions. $P < 0.05$ is considered significant.

## RESULTS

### The results of acquired application pressures

All injections had characteristic slow increase of pressure during the very beginning of application (first 10-15 seconds), which then resulted with maximum pressure, which was afterwards followed by significantly lower pressure during the rest of application.

All perineural injections resulted in injection pressures below 40 kPa [Figure 1]. In contrast, intraneural injections resulted in significantly higher peak pressures ($P < 0.01$) [Figure 2]. In seven (70%) intraneural injections, the injections pressures were over 140 kPa (140-350 kPa).

It has to be noted that out of 10 intraneural injections, 7 injections resulted with high injection pressure (>140 kPa) and the other 3 intraneural injections have resulted with lower injection pressure (<40 kPa).

### Results of neurological examination of experimental animals

All pigs have shown signs of blockade of sciatic nerve after awakening from general anesthesia. The motor function recovered completely during the observation period in all injections joined with lower injection pressure (<40 kPa). To be more precise, all hind limbs of animals with perineural application of local anesthetic had regular neurological status 4 hours after application.

All intraneural injections joined with lower injection pressure showed significantly longer recovery of sensory-motor functions, which came about at the end of experiment. Neither one of extremities, which had perineural or intraneural injection joined with low injection pressure, have shown residual paresis, ataxia, or absence of reaction to pain sensations at the end of study.

Contrary to that, within all intraneural injections joined with high injection pressure (>140 kPa), was detected serious and persistent motor deficit of hind limbs ($P \leq 0.05$) every day during the monitoring of the animals, as well as at the end of the experiment. Additionally, all extremities from the group of high injection pressure (7/10) showed signs of medium to severely expressed paresis, ataxia, or absence of reaction to pain sensation at the end of the study [Figures 3-5].

To determine whether neurological parameters are collinear regarding the grade of defect, medium value of correlation during specific time has been examined separately between perineural and intraneural treated group. Using paresis as basic measure of injury we have examined the time period of reappearance of paresis for 4, 24, and 168 hours after the procedure in groups defined with low application pressure ($P < 40$ kPa), and high injection pressure ($P > 140$ kPa). In the group with high injection pressure after 4 hours the paresis was more frequently evaluated as flaccid extremity or severe paresis, while 7 days after the procedure paresis was evaluated as severe paresis rather than moderate paresis. On the contrary, the group with low injection pressure after 24 hours did not show signs of paresis.

After executed neurological exam, it has been established that all intraneural injections joined with high application pressure resulted with failings, which lasted more than 24 hours, and neurological deficits were evident yet at the end of experiment, after 7 days, which clearly shows that intraneural injection caused the nerve damage.

On the contrary, all injections combined with low injection pressure, whether they were intraneural or perineural did not result with neurological sequels at the end of experiment. Furthermore, in most cases neurological deficit has withdrawn within first 24 hours of experiment.

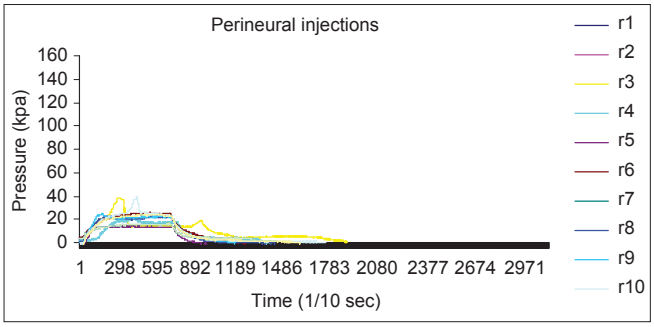


**Figure 1:** Perineural applications

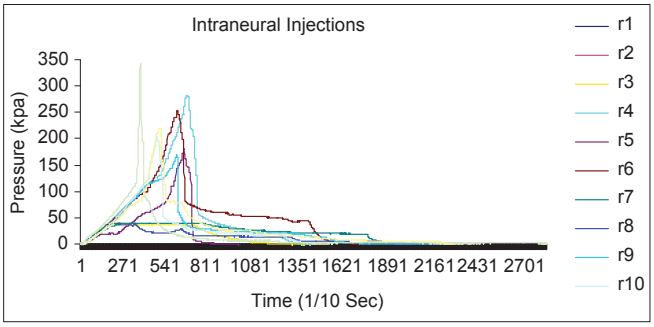


**Figure 2:** Intraneural applications

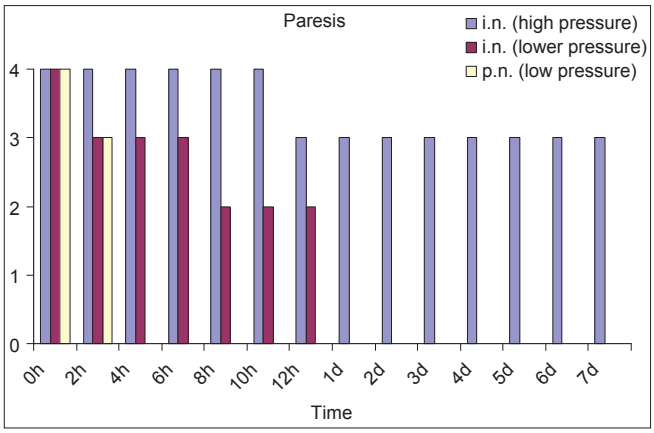


**Figure 3:** Paresis of hind limb after injection application of 2% of lidocaine

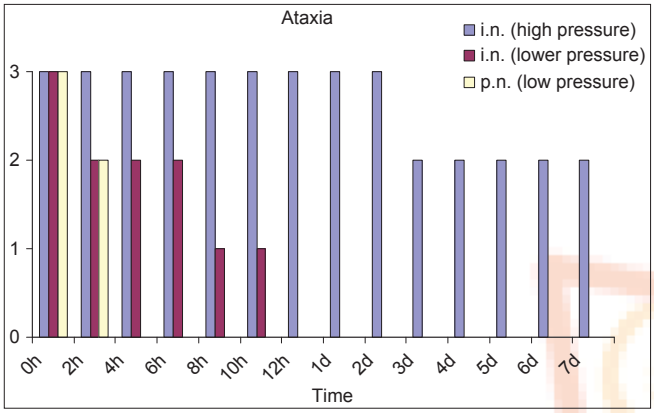


**Figure 4:** Ataxia of hind limb after injection application of 2% of lidocaine

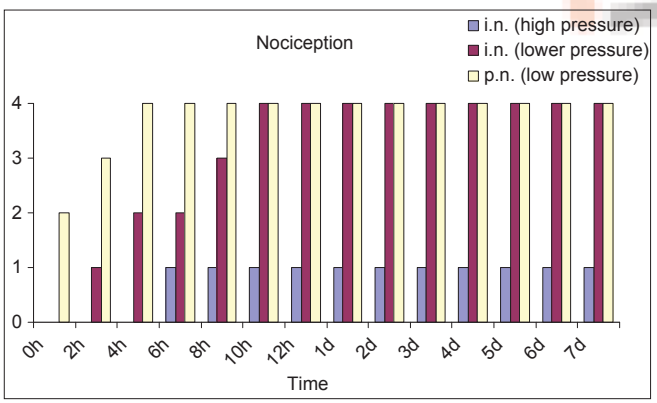


**Figure 5:** Nocioception of hind limb after injection application of 2% of lidocaine

## DISCUSSION

Intraneural injection of local anesthetic in sciatic nerve block model in pigs is associated with high injection pressure (>140 kPa) and persistent neurologic impairment. This finding supports the concept of poor compliance of nerve fascicles and suggests that injection pressure > 140 kPa can be used as a marker of intraneural injection.

Neurologic injury is potential complications of blockades of all peripheral nerves, and methods of prevention of intraneural injections are essential in regional anesthesia. The potential harmful effect of intraneural injection has been acknowledged for over 30 years.[6] It is a debated topic in the current literature. The incidence of long-term nerve damages during peripheral nerve blockades varies between 0.02% and 0.4%, depending on kind of damage and duration of the follow up.[7,8] The incidence of persistent neurological injury is reduced with time passed. Proofs of neurological abnormality occur in 19% of patients within first 24 hours, and decrease to 3-8% through 4-6 weeks, and fall to 0.05% within a year.[9]

From the previous studies it is known that all local anesthetics are potentially neurotoxic if they have been used in overdosed concentration or if they act on nerve through prolonged time period.[10] However, previous experience shows that perineural applied local anesthetic is significantly avoid of 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 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 local anesthetic (appropriate doses and concentrations) 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. In fact, several studies on nerve damages in which various injection solutions were used, showed that only intraneural injection results with nerve damage, which could be registered at earliest 30 minutes after intraneural injection.[11,12] Our results correspond to results from previous studies showing that intraneural injection increases the risk of nerve damage.

The mechanism by which unintentional intraneural injection causes nerve defect is still not known well enough. The mechanism of injury after intraneural injection includes direct mechanical injury, changes in permeability of blood–nerve barrier, joined edema, pressure ischemia, epinephrine-mediated vasoconstriction, and increased pressure of endoneural liquid, which all bring about 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 intraneural injection is mechanical trauma, and that depending on the kind and dose of applied solution and on the addition of epinephrine, we can find various types of nerve damages.[13] Our results show that combination of intraneural application of needle and high injection pressure during the application of local anesthetic can lead to persistent neurological deficit.

An earlier study performed in rabbits has suggested that generally a greater pressure is required to inject solutions intraneurally than perineurally.[14] In the recent studies on dogs and rats, most of intraneural injectios resulted in high injection pressure, which was followed by fascicular injury and motor deficit.[15,16] Indeed, in our study, seven intraneural injections were associated with injection pressure greater than 140 kPa, whereas none of the perineural injection resulted in a pressure greater than 40 kPa. In our study, hind limbs recovered within 4 hours after perineural (normal) injection, which matches "normal" nerve block derived in clinical conditions. However, apparent neurological deficit has been detected at the end of the study with all intraneural injections, which resulted with high injection pressure (>140 kPa, 7 out of 10 injections), which is in accordance with the recently published study.

Previous studies, however, have used small animal models.[16,17] We studied pigs because the risk of permanent injury appears to be greater in large animals and because of their lower regenerative capacity as compared with that of small animals (e.g., rats, rabbits). In addition, previous studies have used clinically irrelevant injection speed or equipment.[16,17] Kapur and Vuckovic used somewhat slower rate of injection (4 ml/min), than what is commonly used clinically (9-27 ml/min). Consequently, the results of those studies remain of questionable relevance to clinical practice. In this study we used equipment and injection rate (15 ml/min) in common clinical use to study the consequences and pressure dynamics of intraneural injection. Because of using high rate of injection speed and because of using larger animals achieved pressure in our study during intraneural application was significantly higher than in previous study mentioned. However, the slope gradient toward the achievement of maximum pressure was the same, indicating that the impulsive increase in pressure during the application leads to the development of nerve injury.

Three intraneural injections did not result in high injection pressure. It is possible that three low-pressure intraneural injections that did not result in neurologic consequences, the needles were not lodged intraneurally but between fascicle, extrafasciculary instead of intrafasciculary. This is because it is difficult to ensure intrafascicular placement of the needle even under direct vision. Animal experiments have been performed to study the significance of intraneural injection. Intrafascicular injection results in worse functional outcome compared with extrafascicular injection in canine model.[17] In a rodent model, Whitlock[18] demonstrated that intrafascicular injection of 0.75% ropivacaine resulted in more severe histological abnormalities compared with extrafascicular injection. High injection pressure may rupture the perineurium, interrupt capillary blood flow and cause neural ischemia. It is obvious that intraneural injection do not always lead to nerve injury. However, high injection pressure during intraneural injection may be indicative of intrafascicular injection and predicts development of neurologic injury.

## CONCLUSION

Detection of pressure during peripheral nerve blocks is unique as a nerve localizing technique in terms of being able to avoid needle–nerve contact and potentially prevent direct trauma to nerves. If these results are applicable to clinical practice, avoiding excessive injection pressure during nerve block administration may help to reduce the risk of neurologic injury. As long as the injection pressure is low, injection into poorly compliant tissue can be avoided and neurological complication can be prevented.

## REFERENCES

1. Cheney FW, Domino KB, Caplan RA, Posner KL. Nerve injury associated with anesthesia. Anesthesiology 1999;90:1062-9.
2. Gentili F, Hudson AR, Hunter D, Kline DG. Nerve injection injury with local anesthetic agents: A light and electron microscopic, fluorescent microscopic, and horseradish peroxidase study. Neruosurgery 1980;6:263-72.
3. Vučkovic I, Hadžić A, Dilberović F, Kulenović A, Mornjaković Z, Zulić I, *et al*. Detection of neurovascular structures using injection pressure in blockade of brachial plexus in rat. Bosn J Med Sci 2005;5:79-85.

4. Chambers WA. Peripheral nerve damage and regional anesthesia. Br J Anaesth 1992;69:429-30.
5. Thalhammer JG, Vladimirova M, Bershadshy B, Strichartz GR. Neurologic evaluation of the rat during sciatic nerve block with lidocaine. Anesthesiology 1995;82:1013-25.
6. Selander D, Brattsand R, Lundborg G, Nordborg C, Olsson Y. Local anesthetics: Importance of mode of application, concentration and adrenaline for the appearance of nerve lesions. An experimental study of axonal degeneration and barrier damage after intrafascicular injection or topical application of bupivacaine (Marcain). Acta Anaesthesiol Scand 1979;23:127-36.
7. Auroy Y, Narchi P, Messiah A, Litt L, Rouvier B, Samii K. Serious complications related to regional anesthesia. Anesthesiology 1997;87:479-86.
8. Buist RJ. A survey of the practice of regional anesthesia. J R Soc Med 1990;83:709-12.
9. Hogan Q, Hendrix L, Jaradeh S. Evaluation of Neurologic Injury After Regional Anesthesia. In: BT F, editor. Complications of Regional Anesthesia. 1st edition. Philadelphia: Churchill Livingstone; 1999. p. 271-91.
10. Kalichman MW, Moorehouse DF, Powell HC, Myers RR. Relative neural toxicity of local anesthetics. J Neuropath Exp Neurol 1993;52:234-40.
11. Reiss W, Shariat AN, Kurapati S, Hadzic A. Intraneural injections. Reg Anesth Pain Med 2011;36:97-8.
12. Gadsden J, Gratenstein K, Hadzic A. Intraneural injection and peripheral nerve injury. Int Anesthesiol Clin 2010;48:107-15.
13. Mornjaković Z, Dilberović F, Cosović E, Divanović KA, Zaciragić A, Kapur E, *et al*. Histological changes of the sciatic nerve in dogs after intraneural application of lidocaine-relation to the established application pressure. Bosn J Basic Med Sci 2005;5:8-13.
14. Selander D, Sjostrand J. Longitudinal spread of intraneurally injected local anesthetics. Acta Anaesth Scand 1978;22:622-34.
15. Hadžić A, Dilberović F, Shah S, Kulenović A, Kapur E, Začiragić A, *et al*. Combination of intraneural injection and high injection pressure leads to fascicular injury and neurologic deficits in dogs. Reg Anesth Pain Med 2004;29:417-23.
16. Vucković I, Dilberović F, Kulenović A, Divanović KA, Voljevica A, Kapur E. Injection pressure as a marker of intraneural injection in procedures of peripheral nerves blockade. Bosn J Basic Med Sci 2006;6:5-12.
17. Kapur E, Vuckovic I, Dilberovic F, Zaciragic A, Cosovic E, Divanovic KA, *et al*. Neurologic and histologic outcome after intraneural injections of lidocaine in canine sciatic nerves. Acta Anaesthesiol Scand 2007;51:101-7.
18. Whitlock EL, Brenner MJ, Fox IK, Moradzadeh A, Hunter DA, Mackinnon SE. Ropivacaine-induced peripheral nerve injection injury in the rodent model. Anesth Analg 2010;111:214-20.

**How to cite this article:** Hasanbegovic I, Kapur E, Kulenovic A, Gojacic A, Dzubur A, Musanovic J, Pepic E. Detection of nerve structures during peripheral nerve blockade in pigs model. Arch Pharma Pract 2013;4:51-6.

**Source of Support:** Nil. **Conflict of Interest:** The authors report no conflicts of interest.