Histological Changes of the Sciatic Nerve in Dogs After Intraneural Application of Lidocaine – Relation to the Established Application Pressure

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Abstract

Histological changes of sciatic nerve in adult dogs 7 days after single application of 2% lidocaine (4 ml dose, speed of injection 3 ml/min) and measurement of the application pressure was studied, with a goal to investigate structural changes of the nerve in relation to the established pressure values. The application pressure was determined by using Bio Bench software. In intrafascicular puncture an average application pressure of 198.23 ± 52 kPa was found, and in interfascicular puncture its average value was 53.3 ± 17.9 kPa, with a note that individual differences are regularly present. Seven days after the injection, a nerve dissection was performed and serial sections covering the region of injection's puncture and bordering proximal and distal zones, in the total length of 3 cm, were prepared. The found changed show the presence of nerves' fibers lesions with a strong reactivity of Schwann's cell, as well as the change of interstitial structure concerning hypercellularity and occurrence of cellular extravasation. The covering system of the nerve in the zone of epineurium manifests changes of inflammatory process and in perineurium a decomposition of lamella layers and the alteration of their tinctorial properties were noticed. A comparison of the found nerve reactivities in intra- and interfascicular application showed their one-way alteration, although the lesions were more noticeable in the conditions of intrafascicular application. The damages were mostly expressed in the zone of local application of anesthetic, than distally from it, while the damage to the structure in the proximal part is of the smallest degree.

KEY WORDS: intraneural application of local anesthetic, application pressure, histology, dog

Introduction

Peripheral nerves are supplied with blood through an internal network of endoneurium blood vessels and an external network of epineurium blood vessels. These two networks anastomose via transperineureal blood vessels. An intact blood-nerve barrier is important for the preservation of internal nerve milieu. Accumulation of the liquid within endoneural space in different pathological conditions results in an increased endoneural pressure, reduction in the blood-nerve flow and increase in the permeability for osmotically active macromolecules. Regional anesthesia represents a significant problem from the point of nerve damage, especially the damage to the blood-nerve barrier. Carriers of the negative effect are: anesthetic itself, needle's diameter, speed of injection, volume of the injected substance and region of application (1, 2, 3, 4, 5). A strong pain that patient feels is a sign that the nerve was targeted with the needle during the performance of regional anesthesia. That is why it is suggested that the nerve blockage is not performed in anesthetized or deeply sedated patients, because they cannot feel the pain during intraneural application after which nerve damage occurs. High pressure during the application of local anesthetic, as a sign that the needle was introduced intraneurally, can have applicable clinical value which could reduce the risk of neurological injuries (6). In spite of their wide use, there are a surprisingly small number of studies dealing with neural toxicity of locally applied anesthetics (7).

Material and Methods

For this research, we used 9 sexually mature male dogs, of mixed breed, with average body mass of 15 kg and average age 2-3 years. The dogs were kept in a standardized laboratory conditions. Before the experiment was performed, a premedication of animals with acepromazine (0.5 mg/kg, intravenously), atropine (0.04 mg/kg, subcutaneously) and ketamine (5 mg/kg, intramuscularly) was done. In general endotracheal anesthesia (halothane) and aseptic conditions, we surgically approached the nerve between biceps femoris muscle and semitendinosus muscle. We bluntly dissected fascias of those muscles, separated the connections with a retractor and found sciatic nerve. At an angle of 45 degrees, we intraneurally placed a 25-gauge needle in the region of nerve, under direct control of optical instrument. By using an automatic infusion pump (PHD 2000; Harvard Apparatus, Holliston, MA) we applied 4 ml of 2% lidocaine (Bosnalijek, Sarajevo) with the speed of 3 ml per minute. The achieved pressure during the application was registered by an in-line manometer (PG 5000; PSI – Tronics Technologies Inc, Tulare, CA) connected to a computer by an analogue-digital converter (DAQ 6023; National Instruments, Austin, TX). The manometer was placed proximally from the needle with which it was connected by a non-distendible tubes (high durometer polyvinyl chloride injection tubing; 84" arterial pressure tubing; Abbot Critical Care System, Abbot Laboratories, North Chicago, IL). The data about the pressure were analyzed by using a software package (BioBench version 1.2; National Instruments, Austin, TX). After the application is done, the skin wound was sutured with an "x" stitch of non-absorbable suture. Seven days after the injection, an intravital excision of the nerve from the area of puncture and bordering proximal and distal zones, 3 cm in length, was performed. Nerve samples were fixated in 10% formalin, embedded in paraffin, sectioned in series and stained with HE method. Prepared histological sections were analyzed under the light microscope with installed digital camera. For the evaluation of the results, standard statistical methods were used. Computer based evaluation of the mean value of pressures is presented as the mean value (X), standard deviation (SD) and standard error of the mean value (SEM). For investigation of significant differences between intrafascicular and extrafascicular group we used the t-test. *P-value* ($p<0.05$) was significant.

Results

Calculated mean values of the application pressure in ***intrafascicular*** application were (198.23 ± 52 kPa) and in extrafascicular application (53.3 ± 17.9 kPa). At the time of lidocaine application in all nerve samples spindle-shape edemas were noticed in the puncture area and in neighboring proximal and distal zones. In the area of application in intrafascicular injections, changes in the histological structure were noticed, including: *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. Blood vessels incorporated in its structure and its surroundings are more noticeable but there are no signs of hyperemia and extravasation (Figure 1). Nerve fibers of the fasciculus into which the anesthetic was directly injected showed damages in general, while subperineural ones had more intensive changes of wide range

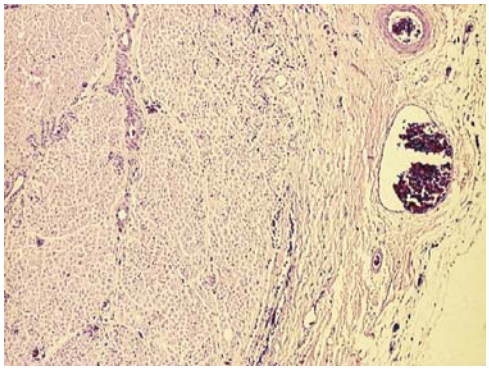

FIGURE 1. Intrafascicular application. HE, x 40.

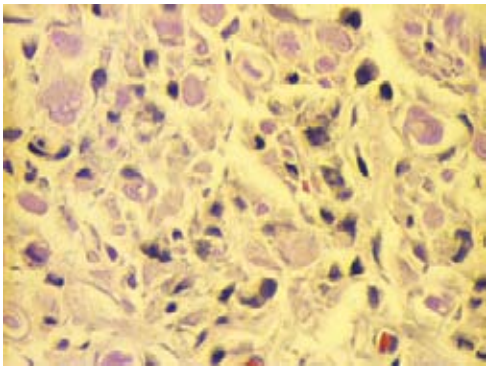

FIGURE 2. Intrafascicular application. HE, x 400.

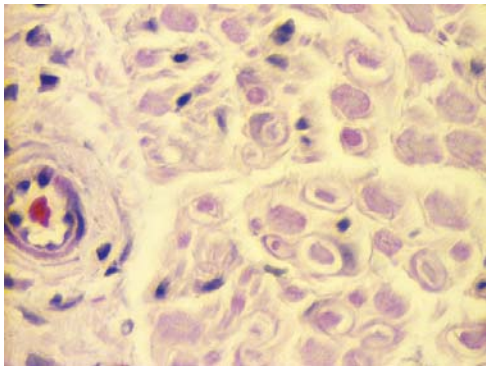

FIGURE 3. Intrafascicular application. HE, x 400.

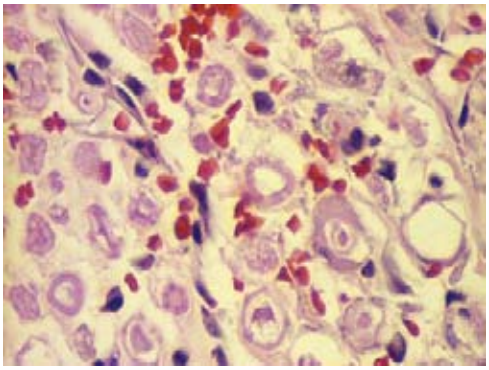

FIGURE 4. Intrafascicular application. HE, x 400.

compared to the central ones. Myelin fibers were disarranged in the space and of increased volume. Some of the axons of these fibers were dislocated and hyperacidophile. In some of them, an advanced axolysis up to the degree of complete disintegration was noticed. In those cases, residual structureless masses appear in fiber's structure. In some areas, myelin sheath showed increased acidophilia and, occasionally, loss of normal structure and transformation into a thin, hyperacidophile, structureless ring. *Amyelin* fibers were disarranged and filled with hyperacidophilic axoplasm (Figure 2, 3, 4). *Schwann's* cells (Figure 2, 3) in the structure of myelin fibers are hypertrophic and some of them noticeably stick out into the interstitium. Consequently, one gets the impression of their separation from the fiber structure. At the same time, their cytoplasm and enlarged nuclei have an extraordinary affinity for the stain. Schwann's cells in the structure of amyelin fibers have less hyperchromatic nuclei. Apart from the above described changes within the fasciculus, a *hypercellularity* can be noticed (Figure 1, 2), contributed by both increased number of Schwann's cells and the mobile cells of the connective tissue, especially macrophages. Intrafascicular blood vessels show hypertrophic endothelium (Figure 3). Individual or grouped extravasally located erythrocytes can be seen in some areas (Figure 4). *Epineurium* (Figure 1, 5) shows hypercellularity of the mononuclear inflammatory process type, with an increased number of macrophages, lymphocytes, plasma cells, hyperemic blood vessels and bundles of collagen fibers with altered, i.e. uneven tinctorial attributes. Collagen fibers are more compactly arranged in the perifascicular zone and strongly acidophilic. In the nerve zone which is located distally from the puncture area as well as in the area proximally from the puncture site, histological changes are of the same

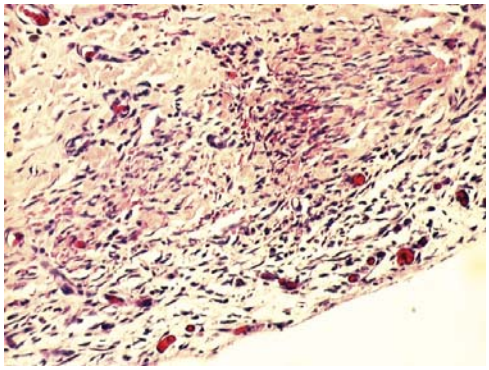

FIGURE 5. Intrafascicular application. HE, x 100

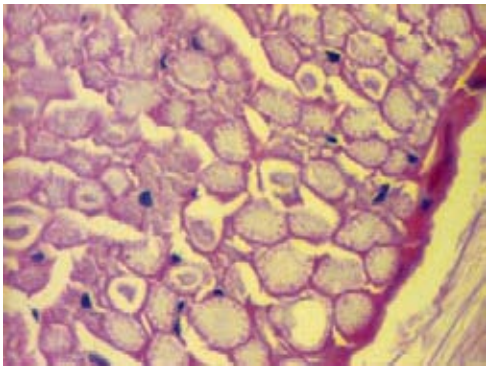

FIGURE 6. Intrafascicular application (proximal segment). HE, x 400.

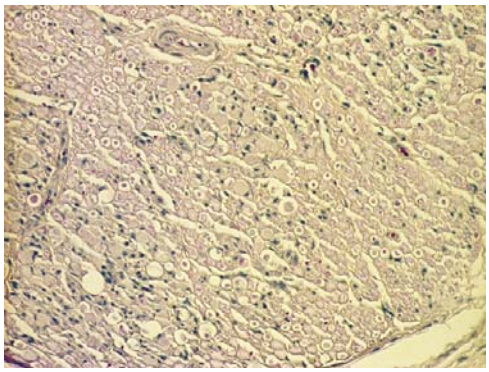

FIGURE 7. Intrafascicular application (distal segment).). HE, x 100.

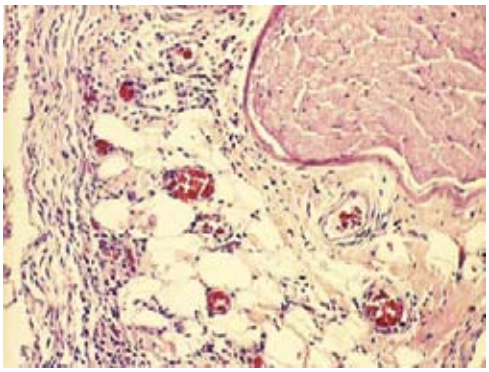

FIGURE 8. Extrafascicular application. HE, x 100.

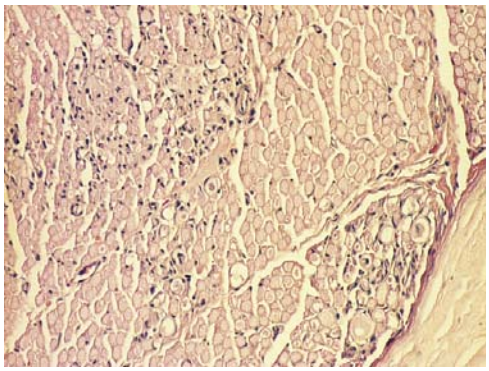

FIGURE 9. Extrafascicular application. HE, x 100.

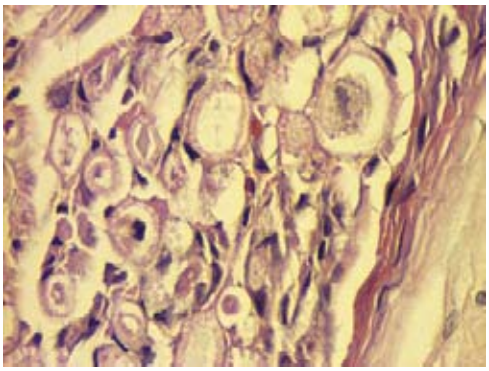

FIGURE 10. Extrafascicular application. HE, x 400.

type but of lower intensity compared to the puncture area (1 cm), especially in the proximal part (Figure 6, 7) In the nerves in which lidocaine was injected ***intraneurally and extrafascicularly***, connective tissue in the structure of epineurium and perifascicular region show hypercellularity of the mononuclear inflammatory process type, so an increased number of macrophages, lymphocytes and plasma cells are registered. Groups of adipocytes with hyperemic blood vessels and bundles of collagen fibers with uneven tinctorial attributes are seen. Intrafascicularly, *subperineural* nerve fibers are more voluminous and Schwann's cells are large with hyperchromatic nuclei. Occasionally, zones of *hypercellularity*, which are the result of the outstanding of Schwann's cells and especially macrophages, are evident (Figure 8, 9, 10).

Discussion

In rare cases, clinical use of local anesthetics is related to neurological morbidity (2). The mechanism of the occurrence of neurological sequelae after intraneural application of local anesthetic is not completely clarified. Some authors state that the degree of nerve damage after intraneural application of various agents depends on the type and dose of the agent (4, 5, 8). Nerve damages during intrafascicular application of local anesthetic are results of direct trauma during application (7) or of ischemia which leads to the damage of blood-nerve barrier and endoneural edema (1). The pressure, as a factor that contributes to the damage, is mentioned, whereby intraneural application is in relation with different levels of application pressure (6). Other authors were mainly dealing with the difference in nerve damage during paraneural and intraneural application or with the differences in damages during paraneural application of different substances (3, 9). We have observed pressure differences in intrafascicular and extrafascicular application of local anesthetic, keeping in mind the different structure within the nerve itself. Our data show that the degree of nerve damage is higher in intrafascicular application which is in relation to high application pressure, with mean values found by us were 198.23 ± 52 kPa, while in cases of extrafascicular application those mean values were 53.3 ± 17.9 kPa, with parallel smaller damages of histological structure. Our previous researches point out the difference in pressure as well (6). Under the conditions of paraneural and extrafascicular application, local anesthetic dilutes quickly into the surrounding tissue and its concentration falls with systemic absorption as well, which results in changes that are of significantly weaker intensity when compared to intrafascicular application; changes are dominantly present in epineurium and in subperineural region of the fasciculus and intensity of the changes depend on the dose and type of local anesthetic (4, 5, 9, 12). Our research shows this distribution of changes during extrafascicular application as well. Opposite of the topical application, the consequences of the elevation of endoneural pressure during intrafascicular application are edema and ischemia, which results in weakened flow in the fasciculus, difficult dilution and absorption of local anesthetic and extension of its acting, In cases of intrafascicular application of 2% lidocaine, we found damages at the level of nerve fibers, Schwann's cells, intrafascicular blood vessels, interstitium, perineum 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. Other researchers mention similar changes during intraneural application as well, but not in all cases (6, 8, 10 and 11) because they didn't distinguish between intra- and extrafascicular application, based on the histological nerve structure and the differences in achieved application pressures that are in connection with it.

Conclusions

1. In intrafascicular application of lidocaine, significantly higher application pressure was found compared to the one in extrafascicular application.
2. Intrafascicularly injected lidocaine results in significant lesions in the puncture area, where subperineural changes are more intensive than central ones. Segments that are distal and proximal to the puncture site show one-way changes of weaker intensity,especially proximally.
3. In extrafascicular application of lidocaine, changes in epineurium and subperineurally located fibers are dominant.

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