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# REACTIVE CHANGES OF THE SCIATIC NERVE CONNECTIVE TISSUE SHEATHS FOLLOWING PARANEURAL LIDOCAINE APPLICATION

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*Original research:*

**Abstract**
Peripheral nerve injuries following paraneural anesthetic application may result in permanent nerve damage. Intraneural application is one of the main reasons these injuries occur, but nerve structure alterations were also reported after paraneural applications. Our aim was to determine the extent of the reactive changes of the connective tissue sheaths during paraneural application of lidocaine, since these changes represent important indicators of the actual magnitude of nerve damage. Ten male New Zealand rabbits were randomly divided into the control (n=5) and the paraneural group (n=5). The sciatic nerves in the paraneural group were bilaterally operatively exposed. Lidocaine (2% ) was injected into the mesoneurium using an automated infusion pump (3mL/min). At the end of the experiment, a 1-cm long nerve tissue specimen was exised. Intact sciatic nerves of the 5 untreated rabbits served as control. Qualitative and quantitative histological analyses were performed. Proliferation of connective tissue of the epi-, and endoneurium, delamellation and thickening of the perineural lamellae, dilation of blood vessels and thickening of their endothelium were observed. The volume fractions of the epineural and the endoneural connective tissue of the paraneural group were higher than those of the control group. The endothelial cells of the endoneural and the epineural blood vessels were taller than those observed in the control group of animals. The perineurium was thickened. The paraneural application of lidocaine results in histological changes of the sciatic nerve connective tissue sheaths. Therefore, it is necessary to proper monitor the injection procedure in order to prevent nerve injuries.

**Keywords:** local anaesthetic, injection, sciatic nerve, nerve damage

## Introduction

Local anesthetics are extensively used in everyday clinical procedures (Hasan et al., 2017; Sagir A & Goyal R, 2015). Although relatively uncommon, peripheral nerve injuries following local anesthesia are major complications that lead to motor, sensory or mixed impairment of the damaged nerve (Hadzic et al., 2004; Hasanbegovic et al., 2013; Kapur et al., 2007). Peripheral nerve blocks are frequently used in acute and chronic pain management (Curatolo, 2016; Hasanbegovic et al., 2020) which is a grave concern in the high-performance athletes (Harle et al., 2018). Beside the therapeutic application, the nerve blocks are also used for diagnostic purposes in sports medicine and other fields (Luo et al., Streitberger 2020). Peripheral nerve blocks may result in persistent strenght deficits (Luo et al., 2015). The incidence of permanent nerve damage after peripheral nerve blocks lies between 0.02 and 0.04% (Hasanbegovic et al., 2014). The degree of nerve injury depends on the application site, the type and concentration of the applied anesthetic, and the application pressure (Hadzic et al., 2004; Hasanbegovic et al., 2013; Kapur et al., 2007). The prevention of these injuries is still elusive due to lack of injection monitoring techniques. Sharp pain associated with resistance during anesthetic injection might be considered possible signs of nerve injury (Kapur et al., 2007).

Lidocaine is a medium-acting amino-amide-type local anesthetic (Golzari et al, 2014). It blocks the voltage-gated sodium channels and results in a transitory and reversible block of the nerve conductivity via modulation of action potential propagation (Cherobin AC & Tavares GT, 2020; Hasan et al., 2017). The amino-amide local anesthetics are widely used for pain control after small surgical interventions or invasive medical procedures (Cherobin AC & Tavares GT, 2020; Kim et al., 2020). Lidocaine has short onset-time and

in case used in local anesthesia or for nerve blocks its effect is manifested within several minutes and lasts up to three hours (Dullenkopf A & Borgeat A, 2003).
Nerve fibers are surrounded by connective tissue sheaths that provide the nerve components with vital nutrients and support mechanically the nerve during stretching and compression caused by body movements (Hasanbegovic et al., 2013). Myelin sheath damage and reactive changes of the connective tissue sheaths indicate nerve fiber damage (Rydevik et al., 1976).
Despite the relatively low application pressure during paraneural anesthetics application, this route of administration results in histological changes of nerve structures (Hasanbegovic et al., 2013).
Previous studies on the effects of paraneural applications of local anesthetics on the connective tissue sheats in the peripheral nerve have mainly been limited to findings of the qualitative histological analysis. Thus, the aim of our study was to perform morphometric and stereological histological analyses to further ellucidate the effect of lidocaine on the connective tissue sheaths following paraneural application.

## Material and Methods

In this study, 10 male New Zealand rabbits were used (weighing 2.8 kg on average). The rabbits were randomly assigned into two groups of 5 animals: the control group and the paraneural group. The rabbits in the paraneural group were administered 2% lidocaine bilaterally (N=10) into the mesoneurium of the sciatic nerve. The intact sciatic nerves (N=10) of the untreated group of rabbits served as control.
On the day of the experiment, the animals were premedicated with acepromazine (5 mg/kg intramuscularly) and atropine (0.04 mg/kg subcutaneously), and anesthesized with ketamine (5 mg/kg intramuscularly). After skin incision, the sciatic nerves were exposed by blunt separation of the connective tissue between the biceps femoris muscle and semitendinosus muscle. The injection of 2% lidocaine (4mL) was administered into the mesoneurium using a 26G needle at a 15-30 angle degree. The administration was performed through an automated infusion pump (PHD 2000, Harvard Apparatus, Holliston, MA) at the speed of 3 ml per minute. After injection, the incision was sutured and the animals were under observation the next seven days.
At the end of the observation time, the animals were sacrificed using an overdose of tetracainehydrochloride, embutramide and mebensonium iodide, and a 1-cm specimen of the sciatic nerve was excised. The samples were fixed in formalin, embedded in paraffin and stained with hematoxylin and eosin and Azan for the qualitative and quantitative light microscopy histological analysis. The quantitative histological analysis, performed using Ellipse 3D software (version 2.0.8.1), included measurement of the perineurium thickness and the height of the endothelium of the epineural and endoneural blood vessels. Additionally, stereological analysis determining the volume fractions of the connective tissue and the blood vessels of the endo- and epineurium was carried out, as previously described (Kališnik, 2002).
Statistical analysis was performed with the SPSS software (version 19, 2011, Chicago, IL, USA). Data are presented as mean ± standard deviation. The comparison between groups was performed using Student's t-test. A p-value of less than 0.05 was considered statistically significant.

## Results

### *Qualitative histologic analysis*
### *Control group*

The nerve structures of the rabbits in this group were well preserved, without histological alterations (Figure 1A and B).

### *Paraneural group*

In the paraneural group of rabbits, hypercellularity of the epineurium was observed. The collagen fibers found in this part of the epineurium were variably arranged, ranging from edematous areas with loosly organized fibers to highly compact sites. The blood vessels of the epineurium, lined by a thickened endothelium, were dilated and hyperemic.

*Figure 1. Qualitative histological findings* A. Control group, H&E, 400x; B. Control group, Azan, 400x; C. Paraneural group, H&E, 400x; D. Paraneural group, Azan, 400x

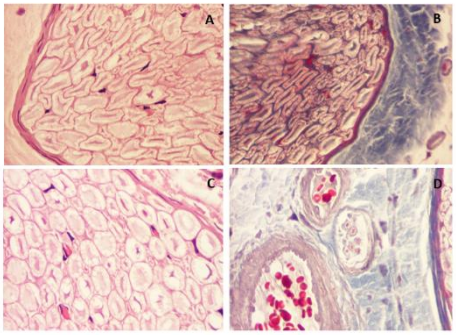

*Table 1. Height of the blood vessel endothelium in the epi- and endoneurium; perineurium thickness*

| Parameter | Control group | Paraneural group | p value |
|---|---|---|---|
| Height of the endothelial cells in the epineurium ($\mu$m) | 1.375±0.159 | 2.563±0.260 | 0,0005 |
| Height of the endothelial cells in the endoneurium ($\mu$m) | 1.285±0.081 | 2.170±0.189 | 0,0005 |
| Perineurium thickness ($\mu$m) | 3.698±0.41 | 5.45±0.92 | 0,0005 |

*Table 2. Volume fractions of connective tissue sheath components*

| Parameter | Control group | Paraneural group | p value |
|---|---|---|---|
| Volume fraction of the epineural connective tissue | 0,407±0,059 | 0,496±0,063 | 0,0005 |
| Volume fraction of the epineural blood vessels | 0.011±0.002 | 0.038±0.008 | 0,0005 |
| Volume fraction of the endoneural connective tissue | 0,153±0,018 | 0,190±0,04 | 0,015 |
| Volume fraction of the endoneural blood vessels | 0.003±0.002 | 0.004±0.001 | 0,154 |

Theperifascicular connective tissue was slightly edematous containing hyperacidophilic collagen fibers of deranged architecture. Larger blood vessels found in the perifascicular connective tissue were dilated and hyperemic.

The perineurium was edematous and its lamellar organization was altered. We also sporadically observed areas with morphologically altered nerve fibers. The endoneural spaces were clearly visible, especially around damaged nerve fibers. The endoneural connective tissue was reactively changed in sense of hypercellularity (due to increased number of fibroblasts and Schwann cells). The collagen fibers of the endoneurium had altered tinctorial properties, and the capillaries were dilated and hyperemic (Figure 1C and D).

### *Quantitative histologic analysis*

#### *Morphometric analysis*

Statistical differences between groups were found in all observed parameters (Table 1).

### *Stereological analysis*

The results of our stereological analysis show a significant increase in volume fraction of the epineural and endoneural connective tissue, as well as the volume fraction of the epineural blood vessels in the paraneural group in comparison to the control group (Table 2).

## Discussion

Peripheral nerves are divided into three compartments: the epineurium, perineurium, and endoneurium (Nadra et al., 2012). The connective tissue located between the epineurium and the structures surrounding the nerve is called mesoneurium (Hasanbegovic et al., 2013).

Although it is not a structural part of the nerve in the way endo-, peri- and epineurium are, it plays an important role in the diffusion of the anesthetic into the epineurium and other structural components of the nerve. Andersen et al. (2012) have shown that it is possible to clearly visualize and identify the paraneural sheath, that appears as a multilamellar circular fascia of loose connective tissue, by histological analysis and/or using ultrasound. The paraneural sheath sporadically contains adipocytes and its thickness varies at different sites. The histological characteristics of the connective tissue sheaths are of crucial importance for the penetration of different substances into different nerve subcompartments. These factors might significantly influence the outcome of regional anesthesia. Peripheral nerve injuries following local anesthesia are rare, but serious complications of anesthetic injections (Hadzic et al., 2004; Hasanbegovic et al., 2013; Kapur et al., 2007).

Additionally, the injected substance might have neurotoxic properties and increase the extent of the damage to nerve subcomponents (Hasanbegovic et al., 2013; Kim et al., 2020; Verlinde et al., 2016).

Therefore, we aimed to determine the histological alterations of the nerve, primarly its connective tissue sheaths, following paraneural local anesthetic injection. In that context, we have qualitatively and quantitatively analysed the connective tissue and the blood vessels of the endo- and epineurium, as well as the thickness of the perineurium. Our findings show that the paraneural application of local anesthetics results in damage of all connective tissue and vascular structures of the nerve. We found that the most prominent alterations were blood vessel dilation, connective tissue proliferation and edema, observed in the connective tissue of the nerve sheaths. The epineurium was hypercellular. The arrangement of

collagen fibers varied, areas of compactly and areas of loosly organized collagen fibers were intertwined. Arterioles, venules and capillaries were hyperemic, dilated and lined by a hypertrophic endothelium. Edema was also observed. The stereological and morphometric analyses confirmed the results of the qualitative histological analysis. Mean values of the volume fraction of the epineural blood vessels, height of their endothelial cells, and also the volume fraction of the epineural connective tissue were statistically significantly larger in the paraneural group than in the control group. These finding might be explained by the proximity of the injection site to this connective tissue sheath and the penetration of large amounts of the anesthetic to this nerve compartment (Hadzic et al., 2004, Mornjakovic et al., 2005).

The perifascicular connective tissue was slightly edematous with sporadically disorganized collagen fibers. Large blood vessels found in the perifascicular connective tissue were dilated and hyperemic. Our results are in acccordance with the findings of previous studies (Hadzic et al., 2004; Mornjakovic et al. 2005). The perineurium was delaminated, composed of hyperacidophilic squamous cells. Its thickness was statistically significantly increased in the paraneural group in comparison to the control group. The connective tissue of the endoneurium was hypercellular, containing a large number of Schwann cells and fibroblasts. The capillary bed was hyperemic and dilated. The volume fraction of the blood vessels in the endoneurium of the paraneural group was higher, but not statistically significantly different to the one of the control group. This might be related to the fact that the perineurium represents a diffusion barrier to the injected substance (Nadar et al. 2012). Application of local anesthetics might disturb the blood-nerve barrier composed of perineural lamellae and endothelial cells of the endoneural blood vessels. The perineurium is the more resistent part of this barrier (Cosovic et al., 2013; Hadzic et al., 2004).

Previous studies have shown that the extent of nerve damage is largely dependent on the application site and pressure, much more than on the concentration of the applied anesthetic (Hasanbegović et al. 2013). This in accordance with the results of our analyses which have shown that the extent of histological changes in the observed parameters of the epineurium of the paraneural group is higher than the extent in the control group, and that the histological changes in the endoneurium are less pronounced than in the epineurium. The contact surface area of the anesthetic and the connective tissue and vascular components of the nerve is extremely large following paraneural applications, resulting in significant reactive changes in connective tissue and vascular structures. Studies show that, after intrafascicular injection, histological changes in the structural parts of the nerve are pronounced, while the extent of these changes following a perineurally administered substance is minimal as the result of lower application pressure (Cosovic et al., 2013; Hasanbegovic et al., 2012; Mornjaković et al., 2005). One would expect that, since injections of local anesthetics into the mesoneurium (paraneural application route) are associated with relatively low values of application pressure (Hasanbegovic et al., 2012), there are no morphological changes of the nerve structures after this route of administration. Our study revealed evidence that runs contrary to these assumptions, the paraneural application results in histological changes of the connective tissue and vascular structures of the endo-, peri- and epineurium.

## Conclusion

The paraneural application of lidocaine induced histological alterations of all connective tissue and vascular structures of the nerve in form of an inflammatory-reparative response. Thus, monitoring of injection is indispensable for prevention injuries following application of anesthetics.

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