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RESEARCH ARTICLE

The Axon Number of the Rat Sciatic Nerve: A Stereological Study

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Summary

This study was designed to determine with stereological methods the number of axons in the sciatic nerve as a result of peripheral nerve blockage following injection of lidocaine and prilocaine into hind limb muscle. Nine adult female Sprague-Dawley rats weighted 150-200 g were used in the study. Ketamine was employed intraperitoneal to the rats as 50 mg/kg. The prilocaine and lidocaine were equally (5 mg/ml) injected to the left and right limbs of the 4 rats. As a control group, isotonic sodium chloride (0.9%) was performed as 0.2 ml into the extremities of the remaining 5 rats. When the application completed, surrounding muscles and soft tissues as well as sciatic nerve were dissected as 0.5x1.0 cm length. In order to determine neurotoxic effects of lidocaine and prilocaine on sciatic nerve, the number of axons was computed by unbiased stereological method. Following of the square root transformation, Mann-Whitney U test was performed to compare groups. When the unbiased comparisons were executed to both groups, the effects of lidocaine and prilocaine on sciatic nerve were found statistically non-significant.

Keywords: *Axons number, Sterology, Rat, Sciatic nerve*

Sıçan Siyatik Sinir Akson Sayısı: Stereolojik Bir Çalışma

Özet

Bu çalışma, Lidokain ve prilokain'in arka ekstremite kası enjeksiyonunu takiben oluşan periferik sinir blokajı sonucunda siyatik sinirde akson sayısını stereolojik metotlarla belirlemek için planlandı. Bu çalışmada 150-200 g ağırlığında dokuz adet Sprague-Dawley tipi erişkin dişi sıçan kullanıldı. Sıçanlara Ketalar 50 mg/kg intraperitoneal uygulandı. Dört adet dişi sıçanın sağ ekstremitelerine eşit miktarda prilokain 5 mg/ml (0.2 ml) ve sol ekstremitelerine lidokain 5 mg/ml (0.2 ml) verildi. Kontrol grubu olarak %0.9 izotonik sodyum klorür kalan 5 adet sıçan ekstremitelerine 0.2 ml uygulandı. Uygulama sona erince çevre kas ve yumuşak dokular siyatik sinir ile beraber 0.5x1.0 cm uzunlukta çıkarıldı. Lidokain ve prilocainin siatik sinir üzerine nörotoksik etkilerini ortaya koymak için tarafsız stereolojik metod ile akson sayısı hesaplandı. Bu özellik bakımından denek ve kontrol gruplarını karşılaştırmada karekök transformasyonu yapıldıktan sonra Mann-Whitney U testi kullanıldı. Her iki grup arasında yapılan karşılaştırma tarafsız stereolojik hesaplamalarla yapıldığında sıçanlara uygulanan Lidokain ve prilokain akut olarak siyatik sinir akson sayılarına etkileri istatistiksel olarak anlamlı bulunmadı.

Anahtar sözcükler: *Akson sayısı, Stereoloji, Rat, Siyatik sinir*

INTRODUCTION

Regional anesthesia with a nerve -blocking is a useful technique for day-stay surgery to the hand [1]. Regional anesthetic techniques provide efficient postoperative analgesia [2,3]. The most often used local anesthetics for regional anesthesia in Europe is prilocaine and lidocaine. They have a relatively short duration of action and are the least toxic of the amino-amide local anesthetics [4,5]. Peripheral nerve injury is a rare but sometimes has the devastating complication of regional anesthesia.

Neurotoxicity of local anesthetics *per se* is not new although it is largely ignored by clinicians [6]. Laboratory

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evidence suggests that local anesthetic neurotoxicity is at least partially dose and concentration dependent [7]. Studies of local anesthetic-induced nerve injury have eliminated several possible mechanisms of toxicity. For example glucose included in the local anesthetic solution is not neurotoxic which suggests that toxicity results from an action of the local anesthetic itself [8]. Therefore we investigated stereological the acute neurotoxic effects of lidocaine and prilocaine on sciatic nerve using injection technique of continuous peripheral nerve blockade.

MATERIAL and METHODS

Animals

This study was approved by the Animal Use Ethics Commission from Yüzüncü Yıl University (2003/03-01). Nine female rats (150-200 g) were kept in a standard plastic cage on sawdust bedding in an air-conditioned room (22°C) under a 12/12 h light/dark cycle and fed *ad-libitum*.

Chemicals

The animals were anesthetized by ketamine hydrochloric (50 mg/kg) intraperitoneally without muscle relaxation and placed in prone position. The rats were separated as a control (n=5) and experiment (n=4) groups. Under the general anesthesia by using peripheric nerve-stimulator (2 Hz and 0.3 ms B/Braun Stimuplex) and a nerve stimulator needle (22 G x 2-0.7 x 50 mm) skin was incised. Proximal hindlimb muscle and sciatic nerve were exposed. The nerve catheters (20 gauges) were inserted into the sciatic nerve sheaths and fixed bilaterally into two extremities along the sciatic nerve trace. After this procedure skin and subcutaneous tissue were closed by single stitches.

Four animals after placing the catheters were administered lidocaine (5 mg/ml) in left side and prilocaine (5 mg/ml) in right side with a rate of 0.2 ml for a total period of 6 h by opening 1 mA. In control group (n=3) the animals were treated with normal saline by same rate and doses on both sides. After six hour from this procedure, the sciatic nerve with 0.5×1.0 cm volume was cut out with surrounding tissues by removing the stitches and withdrawing the catheters. The animals were sacrificed.

Histological Analysis

The entire sciatic nerves were removed bilaterally en bloc from all rats. The nerves were stretched to in situ length by pinning onto a card and then fixed with 2.5% glutaraldehyde in 0.1 M sodium cacodylate (pH 7.4) for 4-6 h in 4°C. After fixation nerve samples about 0.5 cm long that were taken from the nerves were rinsed in 0.1 M sodium cacodylate (pH 7.4) twice. Specimens were post fixed in 1% osmium tetroxide for 1 h dehydrated in an ascending alcohol series and propylene oxide took into for a 50:50 mixture propylene oxide and Epon 812 for 24 h. These procedures were completed by embedding the tissues 48 h in Epon Embedding Kit (Fluka Chemie Gmbt, Swithzerland). Semi-thin and ultra-thin sections (of 1 mm and 90 nm thickness. respectively) were cut by an ultramicrotome (Super Nova Reichert-Yung, Austria) and stained with 1% toluidine blue.

Stereology

Stereological analyses of sciatic nerves were done according to principles described previously [9]. A manual stereological workstation composed of a digital camera (Nikon COOLPX5400, Tokyo, Japan) a manual dial indicator controlled specimen stage and a light microscope (Nikon Microphot-FX Tokyo. Japan) was used for axon number counting [10]. To obtain an estimation of total myelinated axon number in an unbiased manner the axon profiles in the nerve cross-section are sampled with equal probability regardless of shape size orientation and location that means each sampled item was selected with a systematic random manner [11]. For this aim we used an area fraction approach in the application area of unbiased counting frame was 900 mm^2. A counting frame was placed on to a monitor and the sampled area was chosen by a systematic uniform random manner via dial indicator controlled specimen stage. Meander sampling of each sectioned nerve profiles was done in 70 µm-70 µm step size in a systematic-random manner. This ensures that all locations within a nerve cross-section were equally represented and that all axon profiles were sampled with an equal probability regardless of shape size orientation and location [12,13].

Descriptive statistics were presented as Mean, Median SD, Minimum and Maximum values for axon count. After square-root transformation Mann-Whitney U test was used to compare treatment and control groups for this trait 5% values was considered to statistical significant; MINITAB statistical pocket programmed was used for all statistical.

RESULTS

In our study axon number of sciatic nerve was observed on histological sections from the region applied isotonic injection in both treated and control groups. The neurotoxicity was not observed in both lidocain and prilocain group statistically (P>0.05) *(Table 1, Fig 1)*.

DISCUSSION

Peripheral nerve blockade with a local anesthetic provides excellent pain relief but its clinical utility for the treatment of acute or postoperative pain is sometimes limited by a short duration of effect [14,15]. After placing the nerve catheter it is important to provide fast onset of the block to improve the workflow within the operating theatre. This aim can be reached by using a drug with fast onset [16].

Table 1. *Descriptive statistics of the axon number of the groups treated and control (P>0.05)*
Tablo 1. *Denek ve kontrol gruplarının akson sayılarına ait tanımlayıcı istatistik (P>0.05)*

Groups	N	Mean	SE Mean	StDev	Minimum	Median	Maximum	CV
Lidokain	4	59.74	3.67	7.34	52.70	58.10	70.07	0.1228
Control Left	5	69.53	5.38	12.03	55.46	76.93	80.32	0.1730
Control Right	5	70.09	4.12	9.20	58.75	71.42	83.19	0.1313
Prilokain	4	54.20	3.59	7.17	47.82	52.25	83.19	0.1323

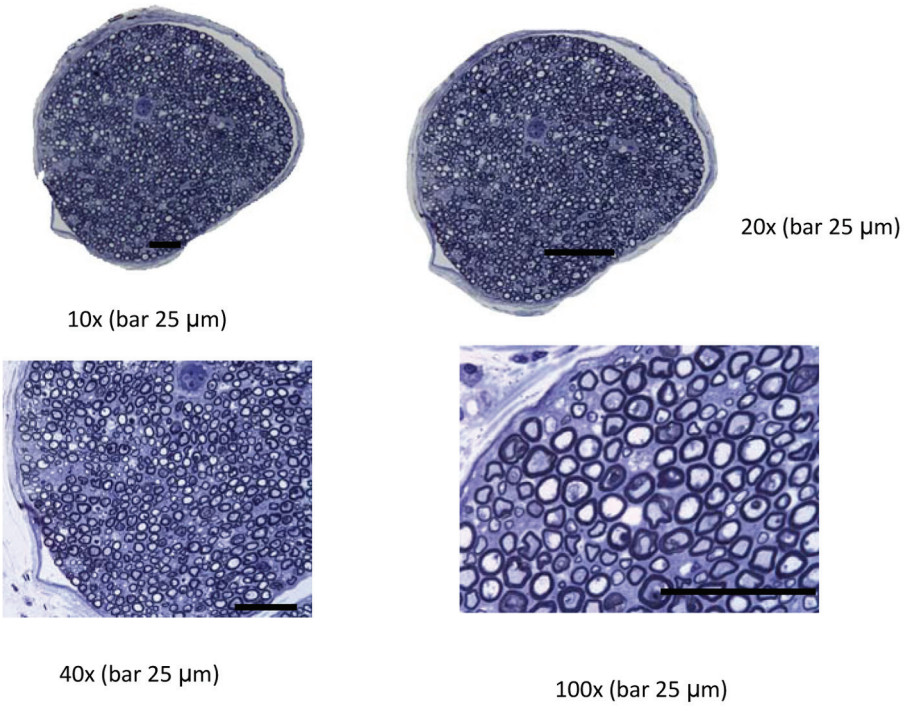

Fig 1. Cross-section of sciatic nerve

Şekil 1. Siyatik sinirin enine kesiti

Local anesthetics are a group of drugs defined by their ability to prevent sodium entry into axons thereby preventing the generation of propagated action potentials in axons. However they have other actions such as prevention of axonal sprouting and effects on G-protein-coupled receptors and on conductance of ions in addition to sodium that might be important in the management of pain [17]. The transport of the amide local anesthetics such as lidocaine and prilocaine is by passive diffusion this transport is dependent on the pH across biological membranes in the case of the amide local anesthetics. Higher concentrations of the anesthetic will be found in the tissue or compartment with lower pH [18].

Prilocaine is medium-long-acting local anesthetics with a fast onset of action [16]. Prilocaine stabilizes the neuronal membrane by inhibiting the ionic fluxes required for the initiation and conduction of impulses thereby effecting local anesthetic action. Local anesthetics bind selectively to the intracellular surface of sodium channels to block influx of sodium into the axon [19].

Studies of clinical adverse effects of intrathecal local anesthetics suggest cauda equina syndrome results from local anesthetic neurotoxicity [20]. Lidocaine toxicity is not the result of an immediate and irreversible breakdown in the integrity of the plasma membrane as would be reflected by a rapid and permanent loss in membrane potential. The mechanism of the lidocaine-induced depolarization currently is unknown but may reflect the simultaneous blockade of ion channels and pumps responsible for the maintenance of neuronal resting potential [21].

Peripheral nerve injury is occurs as a complication of regional anesthesia. Ischemia is one of the causative mechanisms. This may result from changes in peripheral blood flow caused by the local anesthetic itself and/or a vasopressor adjuvant. Peripheral nerves have a dual blood supply of intrinsic exchange vessels in the endoneurium and an extrinsic plexus of supply vessels in the epineurial space that crosses the perineurium to anastomose with the intrinsic circulation. The extrinsic supply is known to be responsive to adrenergic stimuli [22].

Persistent reductions in peripheral nerve blood flow (NBF) can lead to pathological changes in the structure of nerve fibers and their supporting cells [23].

The intravenous regional anesthesia (IVRA) is a technique suitable for hand and forearm surgery and

safe when performed with care by expert practitioners. The most often used local anaesthetic for IVRA in Europe is prilocaine. It has a relatively short duration of action similar to that of lidocaine [4] and is the least toxic of the amino-amide local anesthetics [5].

Although the axonal membrane repolarizes after wash of lidocaine an irreversible conduction block was observed in axons after 3 min exposure of the axon to lidocaine at concentrations as low as 40 mM [24]. Concentration dependence of lidocaine induced irreversible conduction loss. That we observed recovery of soma action potential after exposure of DRG neurons to even higher concentrations of lidocaine raises the possibility that different mechanisms underlie neuronal death and loss of conduction. Because an increase [Ca^{++}] can disrupt cytoskeleton [25,26] and disruption of cytoskeleton can decrease excitability [27]. Lidocaine-induced increase in [Ca^{++}] possibly could contribute to both cell death and long-lasting conduction failure. Regional anesthesia-induced nerve injury may in fact require a combined mechanical and chemical insult [28].

In rat sciatic nerve relative neural toxicity and relative motor nerve conduction blockade were assessed for two amide-linked local anesthetics (etidocaine and lidocaine) and two ester-linked local anesthetics (chloroprocaine and procaine). The nerve fiber injury and edema were assayed by light microscopic examination of nerve tissue sampled two days after perineural (next to the sciatic nerve) injection of various concentrations of the local anesthetics. Both nerve injury and edema increased with concentration of local anesthetics but injury was frequently present in nerve fascicles with little or no edema. In previous studies the amplitude of the electrical activity elicited from the interosseous muscles of the foot following ipsilateral electrical stimulation at the sciatic notch was monitored for up to are minutes to assess the extent of motor nerve blockade. The resulting low concentration-response curves were analyzed for differences in potency. Both for injury and for conduction block the order of decreasing potency was etidocaine, lidocaine, chloroprocaine and procaine. These results are not consistent with the proposal that ester-linked agents are more likely than other local anesthetic agents to cause nerve injury [29]. With specific regard to lidocaine neurotoxicity an irreversible block of impulse conduction was induced in the frog sciatic nerve within a clinical concentration range of 1% to 2% lidocaine [24]. In rats increasing doses of intrathecal 5% hyperbaric lidocaine resulted in persistent sacral sensory deficits associated with motor weakness [30]. All axons counts were based on the gold standard [31] and fractionators technique [12,32].

In conclusion we found that the comparison between the infusion of lidocaine and/or prilocaine and the placebo showed no significant difference in the histological axon numbers ($P>0.05$).

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