Effects of levobupivacaine and ropivacaine on rat sciatic nerve blood flow

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Background. Ischaemia is one of the causative mechanisms of peripheral nerve injury, a documented complication of regional anaesthesia. Local anaesthetics per se and/or vasopressor adjuvants may account for changes in peripheral nerve blood flow. The aim of this study was to test the effects of levobupivacaine and ropivacaine in a rat sciatic nerve model with respect to local blood flow and histopathological changes.

Methods. Forty-eight female Sprague–Dawley rats were anaesthetized for left sciatic nerve exposure. After baseline nerve blood flow measurement with a laser Doppler flowmeter, 0.2 ml of one of the following solutions was applied topically to the nerve in a random fashion: saline 0.9%; lidocaine 10 mg ml–1; levobupivacaine 2.5 mg ml–1; levobupivacaine 5 mg ml–1; levobupivacaine 7.5 mg ml–1; ropivacaine 2 mg ml–1; ropivacaine 7.5 mg ml–1; and ropivacaine 7.5 mg ml–1 plus epinephrine 5 µg ml–1; all in saline 0.9%. Nerve blood flow was evaluated at 5-min intervals up to 30 min after local application of anaesthetic solution. Three animals per group were killed for histological evaluation 48 h later. Multiple one-way analyses of variance followed by Scheffe’s post hoc test was used for statistical analysis. P<0.05 was considered significant.

Results. Local anaesthetics at all concentrations tested caused significant reduction in nerve blood flow. The combination of ropivacaine 7.5 mg ml–1 plus epinephrine did not reduce nerve blood flow to a greater extent than ropivacaine 7.5 mg ml–1 alone. Low concentrations of levobupivacaine (2.5 and 5 mg ml–1) reduced nerve blood flow to the same extent as lidocaine 10 mg ml–1. No significant histological changes were observed at 48 h.

Conclusion. Despite acute reductions in peripheral nerve blood flow, significant histopathological changes were not observed in this rat sciatic nerve model after topical application of levobupivacaine and ropivacaine at concentrations relevant to clinical practice.


Keywords: anaesthetics local, lidocaine; anaesthetics local, levobupivacaine; anaesthetics local, ropivacaine; blood, flow; model, rat; toxicity, nerve

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Peripheral nerve injury is a rare but sometimes devastating complication of regional anaesthesia. Ischaemia is one of the causative mechanisms. This may result from changes in peripheral blood flow caused by the local anaesthetic 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.1 Persistent reductions in peripheral nerve blood flow (NBF) can lead to pathological changes in the structure of nerve fibres and their supporting cells; hence, the study of NBF after perineural application has been used for neurotoxico logical screening.2 3

Levobupivacaine (the (S)-isomer of bupivacaine) and ropivacaine are local anaesthetics currently used in clinical practice. When applied intradermally, they both exhibited vasoconstrictive properties over a wide range of concentrations.4 5 Although ropivacaine seems to be devoid of important effects on spinal cord blood flow,5 7 the effects
of these agents on peripheral NBF has never been studied specifically.

The aim of this study was to test the effects of levobupivacaine and ropivacaine (with and without epinephrine) at clinically relevant concentrations in a rat sciatic nerve model with respect to local blood flow and subsequent histopathological changes. For this purpose, we compared in a prospective, randomized manner, the agents mentioned above with lidocaine 10 mg ml\(^{-1}\) and placebo.

**Methods**

After approval by the local animal research committee (San Diego, CA, USA), 48 young, adult, female Sprague–Dawley rats, weighing approximately 225 g, were anaesthetized for nerve exposure with an intraperitoneal injection of a mixture of sodium pentobarbital 50 mg ml\(^{-1}\), diazepam 5 mg ml\(^{-1}\) and saline 0.9% in volume proportions of 1:1:2 respectively. Animals initially received an injection of 0.25 ml 100 g\(^{-1}\) body weight and an additional injection of 0.05 ml 100 g\(^{-1}\) body weight if required during surgery. Normal body temperature was maintained during both surgery and data collection, by placing the rats on a heating pad. All animals were anaesthetized for the same period of time in the same environment and were at the same level of anaesthesia, as judged from clinical signs during surgery. The left sciatic nerve of each animal was exposed by lateral incision of the thigh and reflection of superficial fascia and muscle. Extreme care was taken to avoid direct injury to the epineurial circulation and to the radicular arteries supplying it.

NBF was measured with a laser Doppler flowmeter (Model LDV-1; TSI, Shoreview, MN, USA) using a 0.5 mm diameter fibre-optic probe, as described elsewhere.\(^1\) A clear ultrasound transmission gel (Aquasonic 100; RP Kincheloe, Dallas, TX, USA) was used as an interface between tissue and probe to eliminate light scattering and loss, and to minimize the pressure needed to stabilize the probe in a fixed position above the nerve. By direct visualization of superficial microvessels and quantitative measurements, we determined that application of the probe did not alter resting NBF; even when the probe was placed directly over compressible surface venules. However, care was taken in placing the probe to avoid epineurial microvessels; a vessel-free area on the surface of the nerve was always found for the placement of the probe. This gave some assurance that the flow measurements represented integrated capillary flow within the fascicle rather than surface epineurial flow from larger vessels either supplying or draining the endoneurial plexus.

For each nerve, baseline flow measurements were taken repeatedly over a 10-min period and averaged to obtain a single value. Each animal had stable baseline flows; the standard deviations for each animal during baseline measurements averaged less than 1 flow unit. This value was then used as a reference for all subsequent measurements, which are reported as percentages of the baseline value. Immediately after baseline measurement, 0.2 ml of one of the following solutions was applied topically to each sciatic nerve in an order established by envelope randomization: saline 0.9% (Baxter, Deerfield, IL, USA); lidocaine 10 mg ml\(^{-1}\) (Xylocaine; AstraZeneca, Wilmington, DE, USA); levobupivacaine 2.5 mg ml\(^{-1}\) (Chirocaine\(^6\); Purdue Pharma LP, Stamford, CT, USA); levobupivacaine 5 mg ml\(^{-1}\); levobupivacaine 7.5 mg ml\(^{-1}\); ropivacaine 2 mg ml\(^{-1}\) (Naropin\(^6\); AstraZeneca, USA); ropivacaine 7.5 mg ml\(^{-1}\); and ropivacaine 7.5 mg ml\(^{-1}\) plus epinephrine 5 \(\mu\)g ml\(^{-1}\); all solutions were in saline 0.9%. Thus, each solution injected at room temperature was randomly tested on six nerves, which then formed a separate experimental group for statistical comparison. Data on NBF were collected by an investigator unaware of the animal’s group assignment, at 5-min intervals for a period of 30 min, after which the flow probe was removed. Thus, the total experimental period of NBF measurement lasted 30 min but was preceded by a 10-min recording period to determine baseline values.

The superficial muscle layer was then sutured with 4-0 silk and the wound closed with metal clips. The animals were allowed to recover for 48 h before the nerves of a subgroup of three animals per group were excised under pentobarbital anaesthesia, immersed in 2.5% phosphate-buffered glutaraldehyde, and processed for light and electron microscopy. Animals were subsequently killed by pentobarbital overdose. All tissue was studied in semithin (2 \(\mu\)m thick) sections with a light microscope and reviewed for neuropathological changes, including extensive oedema, demyelination and axonal injury, by an independent observer.

Statistical comparisons of blood flow data were made at discrete temporal end-points within the experiment. Multiple one-way analyses of variance followed by Scheffé’s post hoc tests were used as appropriate. \(P<0.05\) was considered significant. Data are presented as mean (SEM) for each group vs time.

**Results**

Compared with placebo, all local anaesthetic agents studied—lidocaine, levobupivacaine and ropivacaine—caused significant reductions in NBF regardless of the concentration tested \((P=0.02\) for lidocaine, \(P<0.001\) for all the others) (Fig. 1). Among them, ropivacaine (2, 7.5 and 7.5 mg ml\(^{-1}\) plus epinephrine) produced the greatest reductions in NBF.

Lower concentrations of levobupivacaine (2.5 and 5 mg ml\(^{-1}\)) reduced NBF to the same extent as lidocaine 10 mg ml\(^{-1}\). Levobupivacaine 7.5 mg ml\(^{-1}\) produced a greater decrease in NBF compared with lidocaine 10 mg ml\(^{-1}\) \((P<0.0001)\).

The decreases in NBF were greater in the three ropivacaine groups (2, 7.5 and 7.5 mg ml\(^{-1}\) plus epinephrine) compared with lidocaine 10 mg ml\(^{-1}\) \((P<0.0001)\). Similarly,
NBF in the three groups treated with ropivacaine decreased to a greater extent than in the groups treated with levobupivacaine at low concentrations (2.5 and 5 mg ml\(^{-1}\), \(P<0.02\) and \(P<0.03\) respectively).

The combination of ropivacaine 7.5 mg ml\(^{-1}\) plus epinephrine did not further reduce NBF compared with ropivacaine 7.5 mg ml\(^{-1}\) alone.

The order of magnitude of NBF decrease was ropivacaine 7.5 mg ml\(^{-1}\) and ropivacaine 7.5 mg ml\(^{-1}\)+ epinephrine > ropivacaine 2 mg ml\(^{-1}\) and levobupivacaine 7.5 mg ml\(^{-1}\) > levobupivacaine 2 mg ml\(^{-1}\), levobupivacaine 5 mg ml\(^{-1}\) and lidocaine 10 mg ml\(^{-1}\).

The maximal decrease in NBF at the highest concentration given (7.5 mg ml\(^{-1}\)) was 67.2% for ropivacaine plus epinephrine, 64.2% for ropivacaine and 51.7% for levobupivacaine.

No significant histological changes were observed at 48 h (Fig. 2), although some sections from each group appeared with minor degrees of oedema, which was seen most frequently in the subperineurial space. These findings applied to all groups and were not considered to be severe or of pathophysiological consequence.

**Discussion**

This is the first study to provide evidence of peripheral NBF changes in a rat sciatic nerve model after topical application of ropivacaine and levobupivacaine at concentrations used in clinical practice. Although significant, the reduction in NBF did not result in subsequent histopathological changes suggestive of peripheral neurotoxicity.

As in previous studies, we employed a laser Doppler flow probe to measure NBF.\(^{16}\) The advantage of the laser Doppler technique is that it permits continuous temporal recordings of NBF in animals that have constant blood pressure but otherwise need not be maintained in a steady, equilibrated physiological state.\(^{7}\) In addition, it has been shown to correspond directly with the more quantitative, isotope-labelled tracer techniques.\(^{7}\) The classic Doppler technique only measures velocity, whereas the laser Doppler flowmetry output signal, called flux, is proportional to the
product of the number of moving particles and their mean velocity. The product of these two variables is a measure of blood flow and can be expressed in calibrated units of ml 100 g \(^{-1}\) min \(^{-1}\). However, because in vivo calibrations have not been made for peripheral nerve, it is not possible to express the results in absolute flow terms. Instead, we report our results as a percentage of a baseline value for NBF, which was determined separately for each animal. Using animals as their own controls reduced the likelihood of inter-individual variability confounding our results. The stability and repeatability of the technique is indicated by the steady readings obtained with control solutions.

A number of confounding variables may have influenced our results. To minimize invasive procedures, we did not monitor arterial blood pressure in the experimental animals. Haemodynamic changes in anaesthetized rats may have affected peripheral NBF. However, it has been shown previously that there is less than 10% variation in systemic arterial pressure during the entire procedure and essentially no change in blood pressure associated with topical application of lidocaine in animals anaesthetized with barbiturate.

Increased systemic blood pressure as a result of epinephrine reabsorption would have led to increased NBF. Thus, our measurement of reduced NBF in the group tested with ropivacaine 7.5 mg ml \(^{-1}\) plus epinephrine would have underestimated, if anything, the true reduction in NBF. Our study design did not include testing of sensory or motor block after application of the local anaesthetic solution under direct vision, and the assumption of a complete block was made. Nevertheless, volumes between 0.1 and 0.2 ml of lidocaine injected perineurally have repeatedly been shown to reliably induce complete sensory and motor block in Sprague–Dawley rats.

Normal NBF in rats is in the range of 9–15 ml 100 g \(^{-1}\) min \(^{-1}\), a value comparable with blood flow in cerebral white matter. Theoretically, NBF can be lowered to a level at which normal cell function is depressed. The size of decrease in NBF that may affect function remains unknown. However, in the brain, electroencephalographic changes can be seen after a decrease in cerebral blood flow of about 50% in humans and 66% in the baboon. Similarly, stripping of the epineurium results in a 50% reduction in peripheral NBF and demyelination in nerve fibres located near the perineurium. Although the results of the present study suggest that a comparable reduction in NBF can be induced pharmacologically with clinical concentrations of anaesthetic agents and adjuvants, individual variability and differences between species should be considered. Despite the fact that the human sciatic nerve is much larger than the rat sciatic nerve, it is interesting that the minimal ratio of drug dose to body weight that produces a full block of function seems to be the same for rats and humans. However, analogies of scale must be made cautiously because the nerve fibres and their physiological parameters are dimensionally identical in large and small mammals and are not scaled to length or mass.

In a previous study, performed under identical experimental conditions in the same laboratory, lidocaine 10 mg ml \(^{-1}\) applied topically to the rat sciatic nerve produced a biphasic response in NBF. The initial rise in NBF (lasting for less than 4 min) could not be demonstrated in our study because the measurements were performed at 5-min intervals. However, after this there was a similar degree of reduction in NBF, which persisted for the entire 30-min recording period.

Levobupivacaine produced a dose-dependent reduction in NBF in the present study. Levobupivacaine at similar concentrations had a biphasic effect on skin microvessels, as assessed by laser Doppler imaging, with an initial phase of vasodilatation followed by later vasoconstriction. The extent of these effects is unlikely to have any clinical consequence. It has been shown recently that the initial vasodilatation was abolished by coadministration of epinephrine, thus prolonging analgesia in human skin.

Ropivacaine produced the most remarkable decrease in NBF in our study, which was not potentiated further by the addition of epinephrine. This confirms the intrinsic vasoconstrictor effect of ropivacaine. Previously, the effect of ropivacaine has been studied on spinal cord blood flow and peripheral tissue. Although clinically relevant doses induced only minor changes in spinal cord blood flow in anaesthetised rats, ropivacaine at 10 and 20 mg ml \(^{-1}\) caused a definite reduction. Using the laser Doppler flowmetry technique, several authors found that ropivacaine, in contrast to bupivacaine, has a dose-dependent vasoconstrictive effect on skin blood flow. This is in agreement with the results of in vitro studies on the femoral artery and vein, where ropivacaine was found to contract vessel smooth muscle. This is reflected in clinical practice by complications potentially related to the use of ropivacaine for regional anaesthesia to end organs.

The mechanism of reduction in peripheral NBF produced by local anaesthetics in general remains unknown. The rate of metabolism affects the regulation of blood flow in the central nervous system. Local anaesthetics reduce metabolic rate of the spinal cord, probably as a result of the profound sensory and motor block after spinal application. Thus, it can be speculated that they reduce spinal cord blood flow by lowering the metabolic rate of the spinal cord tissue. This may not apply directly to peripheral nerves, as they lack the sophisticated vascular control mechanisms associated with the cerebral circulation and are relatively resistant to ischaemic injury. It is apparent, however, from experimental studies, that the peripheral nerve may survive hours of ischaemia and that the subsequent recovery of function parallels the restoration of intraneural blood flow.

Although histology and morphometric outcomes correlate poorly with functional results, neuropathology is an integral part of the modern multidisciplinary approach to neurotoxicity research. The lack of histopathological changes at 48 h in our study does not necessarily rule out peripheral nerve dysfunction. It demonstrates only that the significant

### Local anaesthetic vasoactivity

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reduction in NBF did not result in morphological changes 48 h later in this rodent model.

In conclusion, our study findings suggest that, despite acute reductions in peripheral NBF, significant histopathological changes were not observed in this rat sciatic nerve model after topical application of levobupivacaine and ropivacaine at concentrations relevant to clinical practice.

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