

Effects of early and late diabetic neuropathy on sciatic nerve block duration and neurotoxicity in Zucker diabetic fatty rats

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Editor's key points

- Most studies of diabetic neuropathy use rat models of type I diabetes.
- This study used a more relevant model of type II diabetes.
- Sciatic nerve block duration and neurotoxicity were studied.
- Motor block was prolonged in rats with neuropathy, but there was no evidence of nerve injury.

Background. The neuropathy of type II diabetes mellitus (DM) is increasing in prevalence worldwide. We aimed to test the hypothesis that in a rodent model of type II DM, neuropathy would lead to increased neurotoxicity and block duration after lidocaine-induced sciatic nerve block when compared with control animals.

Methods. Experiments were carried out in Zucker diabetic fatty rats aged 10 weeks (early diabetic) or 18 weeks (late diabetic, with or without insulin 3 units per day), and age-matched healthy controls. Left sciatic nerve block was performed using 0.2 ml lidocaine 2%. Nerve conduction velocity (NCV) and F-wave latency were used to quantify nerve function before, and 1 week after nerve block, after which sciatic nerves were used for neurohistopathology.

Results. Early diabetic animals did not show increased signs of nerve dysfunction after nerve block. In late diabetic animals without insulin vs control animals, NCV was 34.8 (5.0) vs 41.1 (4.1) ms s⁻¹ ($P < 0.01$), and F-wave latency was 7.7 (0.5) vs 7.0 (0.2) ms ($P < 0.01$), respectively. Motor nerve block duration was prolonged in late diabetic animals, but neurotoxicity was not. Late diabetic animals receiving insulin showed intermediate results.

Conclusions. In a rodent type II DM model, nerves have increased sensitivity for short-acting local anaesthetics without adjuvants *in vivo*, as evidenced by prolonged block duration. This sensitivity appears to increase with the progression of neuropathy. Our results do not support the hypothesis that neuropathy due to type II DM increases the risk of nerve injury after nerve block.

Keywords: local anaesthetics; nerve block; neuropathy, diabetic; neurotoxicity

Accepted for publication: 21 May 2014

Diabetic peripheral neuropathy (DPN) is a frequent complication of both type I and type II diabetes mellitus (DM), and the most prevalent neuropathy in the Western world.¹ Diabetics undergo surgery more often than non-diabetic patients,² and several surgical procedures for typical complications of long-standing DM, for example, creation of arteriovenous fistula in patients with end-stage renal disease, might be preferably performed under regional anaesthesia.³

However, diabetic neuropathic nerves may be more sensitive to local anaesthetics and their toxicity, and this hypothesis is supported by two lines of evidence. First, regional anaesthesia in diabetic neuropathic patients may be associated with increased risk of neurological injury.⁴ Limited epidemiological evidence suggests higher risk of neurotoxicity in diabetic

neuropathic patients,^{5,6} even if experimental evidence has been equivocal.⁷ Secondly, DPN may influence nerve block duration.⁴ Clinical^{8,9} and experimental^{10,11} evidence suggests that block duration may be prolonged in diabetic neuropathic nerves. However, most studies were carried out in models of streptozotocin-induced type I DM, which does not reflect clinical reality, in which the huge majority of patients suffer from type II DM.^{5,7}

Our aim was to determine the impact of regional anaesthesia in DPN in an animal model for type II DM. We therefore sought to devise a comprehensive model using behavioural, electrophysiological, and histopathological investigations to determine neurotoxicity of a lidocaine 2% peripheral nerve block and duration of this nerve block in Zucker diabetic fatty

(ZDF) rats with early and advanced diabetic neuropathy, with and without partial glycaemic control. Our working hypothesis was that in a rodent model of type II DM, the presence of advanced (18 weeks) but not early (10 weeks) neuropathy would lead to increased neurotoxicity and block duration after sciatic nerve block with lidocaine when compared with age-matched healthy control animals. The primary endpoint was neurohistopathology 1 week after nerve block.

Methods

The study protocol was approved by the Institutional Animal Care and Use Committee of the Academic Medical Center, University of Amsterdam, protocol number LEICA102868-1. Methods and results are reported according to relevant ARRIVE guidelines.¹²

Animals

Experiments were undertaken in ZDF rats, which were obtained from Charles River Laboratories (L'Arbresle, France). This inbred model of type II DM combines a genetic predisposition (homozygous leptin receptor mutation *fa/fa*, 'diabetic', or heterozygous mutation *fa/+*, 'control') with a dietetic component (Purina #5008 diet, Charles River, L'Arbresle, France).^{11–13} Animals were obtained at 9 weeks of age and were allowed to acclimatize for 1 week. For all electrophysiological measurements, sciatic nerve block, and placement of insulin release

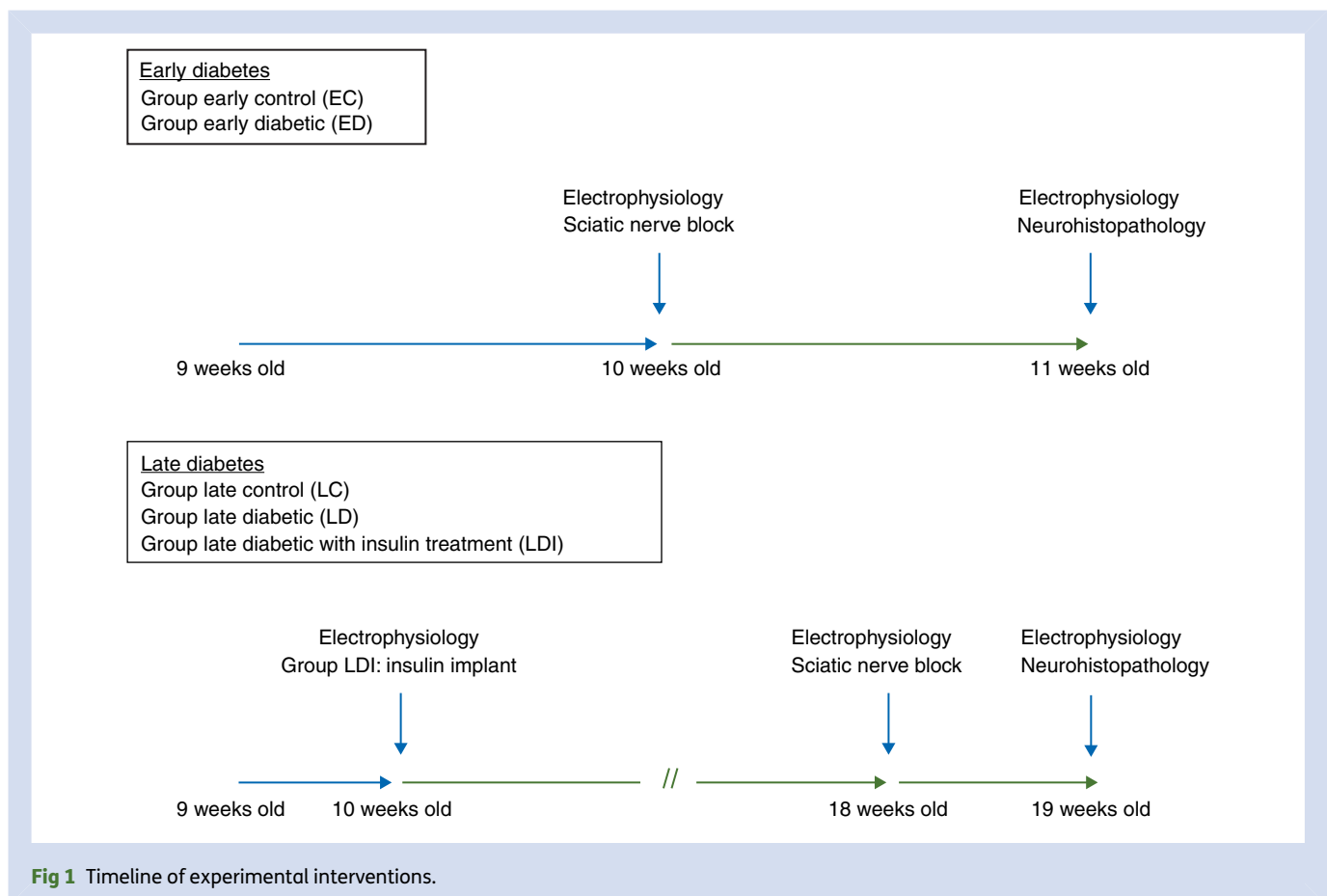
implants, animals were anaesthetized using isoflurane (Baxter, Utrecht, The Netherlands) with an inspiratory concentration between 2 and 3 vol%, since this regimen least affects electrophysiological measurements in rodent models.¹⁴ Adequacy of anaesthesia was ascertained by lack of a pedal withdrawal response to a nociceptive stimulus. All procedures were performed percutaneously, and the analgesic rescue protocol was buprenorphine (0.05 mg kg⁻¹ body weight). Detailed welfare assessment was undertaken by an animal care technician unrelated to the experiment. After the last measurements, while still under isoflurane anaesthesia, animals were killed using CO₂ narcosis.

Experimental groups

The timeline of experimental procedures is given in Figure 1. In all experimental groups, baseline measurements of electrophysiological parameters (see below) were taken at 10 weeks of age.

Group 'early control (EC)' were 6 ZDF *fa/+* animals, and group 'early diabetic (ED)' were 10 ZDF *fa/fa* animals undergoing left sciatic nerve block immediately after baseline testing at 10 weeks. One week later, behavioural and electrophysiological measurements were repeated, and the left sciatic nerve was excised for neurohistopathological evaluation.

The group 'late control (LC)' consisted of 10 *fa/+* animals kept until 18 weeks of age. The group of diabetic animals for



the late experiments were randomized into one of the two groups according to a predefined randomization list: group 'late diabetic without insulin (LD)' were 10 ZDF *fa/fa* diabetic animals kept until 18 weeks of age. Group 'late diabetic with insulin (LDI)' were 10 ZDF *fa/fa* animals, which received 1.5 s.c. insulin implants (LinPlant, LHR-10BV, LinShin, Toronto, Canada) using a custom-made trocar G12-SS, LinShin) at 10 weeks old. These animals were dosed according to weight at 10 weeks, ~300 g, and received ~3 units insulin per day for a period of 60 days, covering our experimental period.¹⁵ Groups LC, LD, and LDI underwent further 'late baseline' electrophysiological testing at 18 weeks of age, followed by sciatic nerve block. One week later, behaviour and electrophysiology tests were repeated, and tissue excision for neurohistopathology was performed.

Serum glucose levels were measured in all animals in blood drawn from the left tail vein, using a commercially available glucose meter (Blue, FIA Biomed, Emsdetten, Germany), which was regularly calibrated. In Groups EC and ED, this was done at 10 and 11 weeks, and in Groups LC, LD, and LDI, this was done at 10, 14, 16, and 18 weeks of age.

Electrophysiology

With temperature maintained well above 34°C using a warming blanket (HK25, Beurer, Ulm, Germany), we studied the sciatic and caudal nerve with monopolar needle electrodes using a Nicolet Viking IVP electromyography system (Nicolet, Madison, WI, USA), as described previously.¹⁶ In brief, for motor conduction studies of the sciatic nerve, the recording cathode was placed in the intrinsic muscles between the hallux and the second digit, and the recording anode was placed subcutaneously on the lateral surface of the fifth digit. Stimulating electrodes were inserted 3 mm apart at the medial ankle, and just cranial to the sciatic notch. A grounding electrode was attached between the stimulating and the recording electrodes. Supramaximal square-wave pulses of 0.1 ms duration were delivered. Supramaximal stimulation was achieved by increasing the intensity by 25–30% above maximal stimulation. Compound muscle action potential (CMAP) amplitudes (peak to peak) were recorded. Motor nerve conduction velocity (NCV) was calculated over the segment between the sciatic notch and the ankle, and minimal F-wave latency was measured from seven F-wave recordings.

For studies of the mixed sensory and motor caudal nerve,¹⁷ recording cathodes and anodes were inserted at the base of the tail just 5 mm apart. The stimulating cathode was placed laterally in the tail at exactly 3 cm from the base of the tail; the stimulating anode was inserted 5 mm distal to the stimulating cathode. The earth electrode was attached halfway between the stimulating and recording electrodes. Supramaximal square-wave pulses of 0.5 ms duration were delivered. Compound nerve action potential amplitudes (negative peak) were recorded. The NCV of the tail nerve was calculated from the latency of the stimulus artifact to the onset of the negative peak of the action potential elicited and the distance between the stimulating and the recording cathodes, which was a

standard distance of 3 cm. All measurements were carried out by one investigator (P.L.), and electrophysiological measurements underwent blinded assessment and validation by an experienced neurophysiologist (C.V.).

Sciatic nerve block

Nerve block was performed percutaneously, combining the technique described by Thalhammer and colleagues¹⁸ modified by nerve stimulation as described by Kroin and colleagues.¹⁰ In brief, a 25 G needle was introduced just caudal to the sciatic notch directed cephalad, and connected with a clip to the Viking electromyography system programmed to deliver a pulse of 0.1 ms duration, and 0.6 mA current, triggered manually. Ipsilateral hind-leg kick in the absence of local stimulation was taken as a sign of proximity of needle to nerve, and injection of 0.2 ml of lidocaine 2% was performed. We defined a successful nerve block on the basis of three signs:

- (i) before injection, successful nerve stimulation at 0.6 mA current,
- (ii) gradual disappearance of the CMAP in electrophysiological recordings after injection of lidocaine, and
- (iii) subsequent behavioural testing showing absence of the toe-spreading reflex.

The latter reflex, used to test sciatic nerve fibres, was tested as described by Kroin and colleagues.¹⁰ Animals were gently lifted, resulting in a physiological vestibular reflex where toes are extended and spread. We noted the presence or absence of these findings to characterize block duration every 15 min until the block subsided. This gross behavioural testing was repeated before the animals were subjected to anaesthesia, 1 week after nerve block to detect any permanent nerve injury.

Neurohistopathology

The main outcome parameter was the nerve injury score of the left sciatic nerve 1 week after nerve block. To this end, after the last electrophysiological measurements, left sciatic nerves were excised immediately after euthanasia. The segment proximal and distal from the site of injection was harvested, and fixed with a 2% buffered formalin solution, embedded in paraffin, cut at 6 µM in longitudinal and transverse sections, and stained with haematoxylin–eosin (HE) and Masson trichrome. Samples were examined under the light microscope for evidence of inflammation in the epineurial, perineurial, and endoneurial compartment, vascular injury, and nerve fibre injury. Nerve fibre injury was assessed using a simple, semi-quantitative four-point score where 0 represents a normal nerve and 4 represents extreme injury with inflammation and destruction of all components of the nerve including axons and myelin, extending throughout the nerve bundle.¹⁹ The pathologist (U.G.) was blinded to experimental group allocation.

Statistical analysis

Semi-quantitative neurohistopathological data such as the primary outcome were compared by the Friedman test followed—if significant—by the Mann–Whitney *U*-test. Power analysis revealed that a group size of 10 animals would have

80% power to reject the null hypothesis that the neurohistopathology score in the one group is significantly different from another group of nerves using the Mann–Whitney *U*-test with a 0.05 two-sided significance level. Body weight, blood glucose level, and neurophysiological data were compared by analysis of variance (ANOVA) between the groups followed by the *post hoc* Bonferroni test for multiple comparisons. Variations of neurophysiological data over time were compared using the paired *T*-tests. A *P*-value of <0.05 was considered significant. Statistical analysis was performed with IBM SPSS® Statistics Version 20 (IBM, San Francisco, CA, USA). Power analysis was done with the aid of nQuery Advisor® 7.0 (Statistical Solutions Ltd, Cork, Ireland).

Results

All animals survived to the end of the experiment, no animal needed analgesic rescue, and no animal fulfilled predefined criteria for termination of experiments (humane endpoints). Welfare assessment showed no abnormalities concerning appearance or behaviour at any time point. All animals showed clinical recovery from sciatic nerve block.

Early diabetes

At baseline (10 weeks), the mean glucose value was 7.1 (1.0) in EC and 13.1 (4.9) mmol litre⁻¹ in ED animals (*P*<0.01). The mean body weight was 288.8 (8.5), respectively, 344.1 (16.7) g (*P*<0.001). In EC vs ED nerves, sciatic nerve MNCV was 39.3 (4.2) vs 35.7 (2.5) m s⁻¹ at baseline (*P*=0.02), and minimal F-wave latency was 7.3 (0.5) ms vs 7.9 (0.6) (*P*<0.005).

Sciatic nerve block duration was 45 (13) min in the EC group, and 67.5 (27) min in the ED group (*P*=0.08).

The differences between electrophysiological parameters at baseline and 1 week after sciatic nerve block were calculated. The mean MNCV was decreased 1 week after block compared with baseline across EC and ED animals (*P*<0.01). There was no difference between EC and ED animals in their change over time. We found no significant differences in electrophysiological CMAP parameters for LC and EC animals at baseline and after nerve block (Supplementary Appendix S1).

In histopathological investigations, most specimens in the EC and ED groups showed mild chronic inflammation in the epineurium and in the adipose tissue. In the EC group, one out of six animals had a minimally elevated nerve injury score of '1+' (out of 4), compared with three out of 10 animals in the ED group with an elevated nerve injury score of '1+' (*n*=1) and '2+' (*n*=2, NS).

Late diabetes

Weight and glucose levels of test animals are given in Table 1. Diabetic animals were randomized at 10 weeks of age to receive insulin treatment (LDI) or no treatment (LD). At 14 weeks of age, the mean glucose values were significantly lower in LDI than in LD animals. However, thereafter the difference was not significant (Table 1). Neurophysiological data of conduction velocity and minimal F-wave latency are given in Figure 2. Conduction velocity increased over time in the LC

Table 1 Body weight and blood glucose. Weight and blood glucose levels of all groups over time. Tested by ANOVA with the *post hoc* Bonferroni correction. NS, not significant; LC, late control; LD, late diabetic; LDI, late diabetic with insulin

Weeks	10	14	16	18
Body weight (g)				
LC (<i>n</i> =10)	282 (14)	364 (17)	386 (23)	402 (37)
LD (<i>n</i> =10)	345 (19)	419 (39)	427 (38)	435 (36)
LDI (<i>n</i> =10)	350 (20)	471 (25)	484 (35)	494 (41)
LC vs LD	<i>P</i> <0.001	<i>P</i> <0.001	<i>P</i> <0.05	NS
LC vs LDI	<i>P</i> <0.001	<i>P</i> <0.001	<i>P</i> <0.001	<i>P</i> <0.001
LD vs LDI	NS	<i>P</i> <0.01	<i>P</i> <0.01	<i>P</i> <0.01
Blood glucose (mmol)				
LC (<i>n</i> =10)	7.6 (1.9)	6.0 (0.5)	6.2 (0.5)	8.1 (1.2)
LD (<i>n</i> =10)	11.9 (5.3)	24.2 (5.1)	25.5 (3.2)	28.4 (3.4)
LDI (<i>n</i> =10)	11.5 (4.6)	17.8 (7.4)	21.6 (5.0)	25.2 (5.5)
LC vs LD	NS	<i>P</i> <0.001	<i>P</i> <0.001	<i>P</i> <0.001
LC vs LDI	NS	<i>P</i> <0.001	<i>P</i> <0.001	<i>P</i> <0.001
LD vs LDI	NS	<i>P</i> <0.05	NS	NS

group, whereas it remained unaltered in LD and LDI animals. The conduction velocity at 18 weeks in the LD animals was significantly lower than in the LC group and remained so for 1 week after sciatic nerve block. After nerve block, conduction velocity tended to decrease in all groups, but this decrease neither reached statistical significance in any of the separate groups nor when data of all groups were pooled (*P*=0.15, Fig. 2A). We found no significant differences in electrophysiological CMAP parameters for LC and LD animals before and after nerve block (data are given in Supplementary Appendix S2). Caudal NCV was slowed significantly when comparing animals in Groups LC [65.3 (12) m s⁻¹] and LD [57.1 (8) m s⁻¹, *P*<0.05].

At 18 weeks, minimal F-wave latencies were significantly prolonged in the LD when compared with the LC group. At 1 week after nerve block, the latency increased significantly, when data from all three groups (LC, LD, LDI) were pooled (Fig. 2B). Decreases in minimal F-wave latency between the three groups were not significant.

Block duration was shortest in the LC group, longest in the LD group, and intermediate in the LDI group (Fig. 2C). The LDI animals had a longer block than the LC animals, but there was no significant difference in block duration between LD and LDI animals. LD animals had a block duration significantly longer [94.5 (33.2) min] than ED animals [67.5 (27) min, *P*<0.05].

In histopathological investigations, we noted only minor changes. LC animals showed minimal, variable, multi-focal chronic inflammatory infiltrates in epineurial connective tissue, generally not extending into the endoneurial or perineurial compartments. One animal out of the LDI group and one animal out of the LD group showed focal oedema or focal area of acute myelin injury and axonal damage, associated with scattered inflammatory cells. The neurohistopathological changes were not different between the groups (Fig. 3).

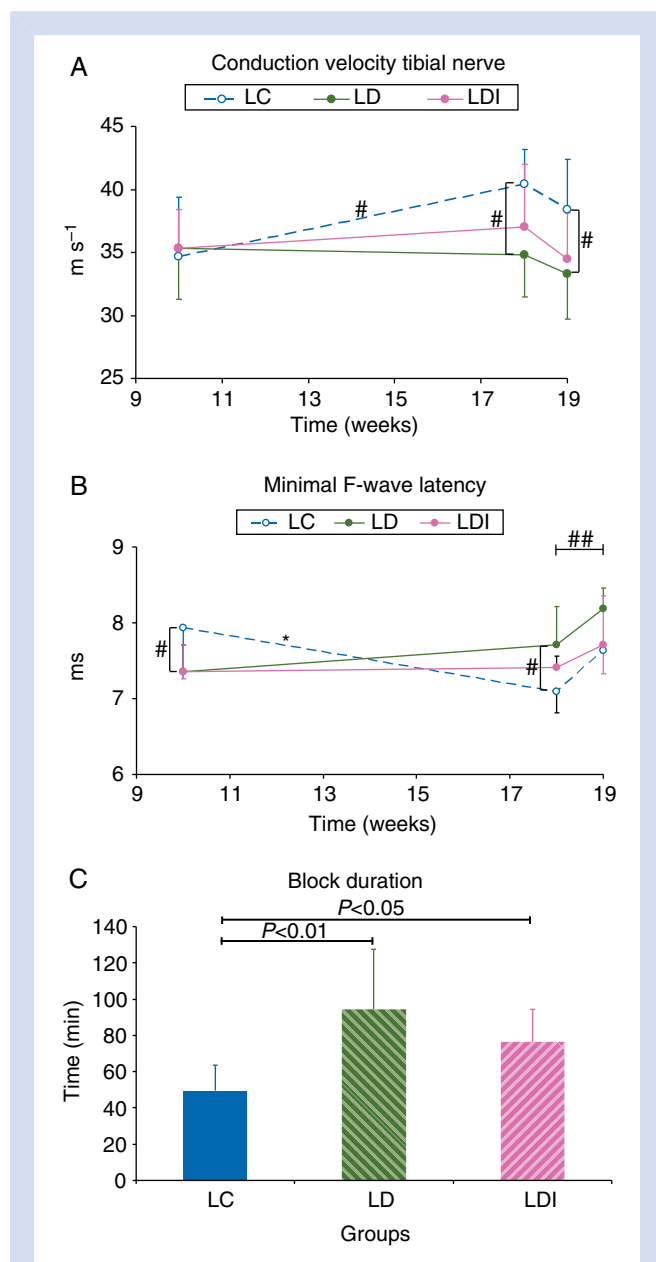


Fig 2 Development of sensible conduction velocity (A) and minimal F-wave latency (B) over time in the three experimental groups. After nerve block, conduction velocity tended to decline in all groups, but did not reach statistical significance when groups were pooled ($P=0.15$). Sciatic nerve motor block duration in late diabetic animals (C). After the nerve block, the minimal F-wave latency increased significantly, when the data of all three groups were pooled. Data are shown as mean and SD. ANOVA with the post hoc Bonferroni test, paired samples T -test. * $P<0.05$; # $P<0.01$; ## $P<0.001$.

Discussion

In ZDF rats at 10 weeks of age, there was no prolongation of duration of sciatic block with lidocaine 2% and no discernible impact on nerve damage 1 week after sciatic nerve block. In older rats (18 weeks), with more long-standing diabetes and clear signs of neuropathy, sciatic nerve block duration was

substantially prolonged, but there were no gross behavioural signs, or increased neurohistopathological evidence of nerve injury 1 week after sciatic nerve block. Electrophysiological changes suggestive of subtle nerve dysfunction after nerve block were present in all experimental groups, unrelated to the duration or severity of the neuropathy.

The ZDF rats are a model of type II DM and therefore represents the predominant patient population more accurately than the previously used STZ-induced type I DM model.⁷ Notably, pathogenesis differs considerably between type I and type II diabetes in experimental models²⁰ and in humans,¹ and implications for regional anaesthesia should preferably be undertaken in a model most closely resembling the clinical situation.⁷ However, all previous investigations had been conducted in models of type I DM *in vivo*,^{10 21-23} or in type II DM *in vitro*.¹¹ Our investigation is the first to investigate the effects of a DPN secondary to type II DM on toxicology and function of sciatic nerve block.

Early diabetes

In our model, ED rats at 10 weeks of age had mildly decreased nerve conduction velocities, indicating mild diabetic neuropathy. The neuropathy in these rats develops over time and our measurements of neuropathy correspond well with previous literature.²⁴ Duration of nerve block was not significantly prolonged ($P=0.08$) in ED when compared with EC animals. We found no significant neurohistopathological or gross behavioural signs of nerve damage 1 week after sciatic nerve block. Our neurotoxicity results correspond to previous *in vitro* investigations in the ZDF model at 12 weeks of age,¹¹ and with an *in vivo* model of type I DM.²³

Late diabetes

LD animals at 18 weeks of age had decreased nerve conduction velocities when compared with LC animals, indicating the development of a more severe diabetic neuropathy over time, concurring with previous literature.²⁵ We found that while there was a slight increase in MNCV in LC over time, the MNCV of LD was not increased. This is in concordance with previous work by Oltman and colleagues,²⁴ where a slight increase in MNCV in lean (healthy) animals over time until the 20th week was observed. Also, we corroborated our MNCV findings by simultaneous measurements of the caudal NCV, confirming diabetic neuropathy also in this predominantly sensory nerve.¹⁷

We found no histopathological signs of increased neurotoxicity in LD animals when compared with age-matched LC animals, after lidocaine-induced sciatic nerve block. Our results mirror previous investigations in type I DM, in which lidocaine at clinical concentrations had limited neurotoxic effects.^{10 21} There have been no *in vivo* local anaesthetic neurotoxicity investigations in type II diabetic models at all, but recent *in vitro* data show only modest neurotoxicity of 2% lidocaine.¹¹ Our data confirm and widen these *in vitro* results using multifaceted testing *in vivo*. Furthermore, epidemiological clinical outcome data suggest that even when long-lasting local anaesthetics are used, nerve injury after neuraxial block in

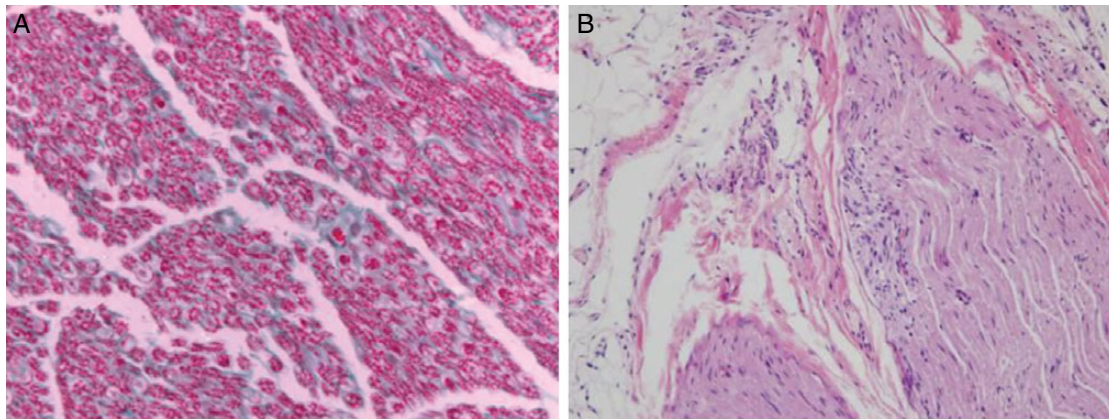


Fig 3 Neurohistopathology of a representative healthy control nerve (A), and a nerve from a representative diabetic animal, showing focal areas of acute myelin injury and axonal damage, associated with scattered chronic inflammatory cells (B).

patients with pre-existing neuropathy is rare. Specifically, in one retrospective study, the incidence of apparent neuropathic complications after neuraxial anaesthesia was two in 325 patients.⁶ Across the past 20 yr, six case reports describing association of nerve damage with diabetic neuropathy after regional anaesthesia have been published.⁵

We note highly significant prolongation of minimal F-wave latency as a subtle marker of nerve dysfunction 1 week after sciatic nerve block. This has not been previously described, but there was no difference between LD and LC animals, such that this most likely represents a minor and unspecific sequel of nerve block. The clinical importance of this remains unclear, and is most probably very limited. There has been discussion whether regional anaesthesia in diabetic patients may induce clinically unapparent damage which may promote progression of diabetic neuropathy.⁷ However, the changes observed here are very small, and need to be investigated in detail before any clinical relevance can be ascribed.

In LD animals, block duration was significantly prolonged, while LDI animals had an intermediate increase in block duration. This finding was expected on the basis of previous findings. Our results differ in magnitude from our previous manuscript, in which several tests were used to quantify motor, deep sensory, and superficial sensory block.¹¹ In comparison, our rather crude ‘on/off’ testing for a vestibular reflex in this study more closely reflects the data obtained by Kroin and colleagues,¹⁰ who used the same method. The main difference with the latter study is that we used 0.2 ml of 2% lidocaine as in our previous study,¹¹ whereas Kroin and colleagues used 0.1 ml of 1% lidocaine.¹⁰ Several studies have assessed block duration in a streptozotocin-induced rat model of type I DM, and while one study found no difference,²¹ three studies showed prolonged block duration in diabetic rats.^{10 22 23} This latter finding was confirmed by us in the ZDF rat model of type II DM.¹¹ Recently, two clinical studies described increased sciatic nerve block duration in diabetic patients.^{8 9} Therefore, the evidence strongly indicates that diabetic neuropathy will

prolong the duration of peripheral nerve block. This prolongation may be caused by pharmacokinetic²⁶ or pharmacodynamic (e.g. modulation of sodium channels by neuropathy)²⁷ mechanisms, but the respective contributions remain unclear. Potential clinical implications are to consider the diabetic nerve ‘more sensitive’ to the effects of local anaesthetics, and it has been proposed to reduce the dose of local anaesthetics when performing nerve blocks for perioperative analgesia.⁴ Also, it had been suggested to reduce or omit epinephrine from peripheral nerve blocks in neuropathic patients,⁴ and the results obtained in experimental and clinical settings would indicate that nerve block duration in diabetic neuropathy will be prolonged anyway, even without the need to add adjuvant epinephrine.

Limitations

In the LDI rats, the effects of insulin were not sufficient to cause glucose levels to be similar to those in control animals, even though the same dose achieved good glucose control in type I²³ and type II²⁸ models of DM. This may be because insulin was dosed according to body weight at baseline (10 weeks), and diabetic animals were substantially heavier at the end of the experimental period, leading to relative under-dosing towards the end of the experimental period, which is supported by the increasing blood glucose levels over time in these animals. Therefore, our insulin regimen was more representative of loose rather than strict glycaemic control.

In diabetic and control animals, small inflammatory changes were noted on neurohistopathological investigation in all groups, which may be explained by repeated stimulation. Sciatic nerve inflammation after repetitive stimulation, as occurred in our study due to neurophysiological measurements, has been described *in vivo* before.²⁹ The timepoint of excision was chosen on the basis of earlier experiments by our group, but differ with the timepoint chosen by Kroin and colleagues¹⁰ (2 days post-block). The mild changes after block were seen both in the study by Kroin and colleagues and our study.

The ZDF model used by us cannot be extrapolated directly to the clinical situation. First, the age of the experimental rat is comparable with that of a young human adult on the basis of physiological and behavioural parameters.³⁰ However, it should be noted that we chose the age of our test animals based on the age at which neuropathy typically develops, which is around 20 weeks.²⁴ Secondly, interspecies differences between rats and humans concerning neuropathy and toxicity of local anaesthetics are unclear. Nevertheless, rodent models have been used to investigate diabetes and its neuropathy,²⁴ and determine functional and toxicological aspects of regional anaesthesia in the past.³¹

Lastly, we attest to the fact that the focus of behavioural testing after sciatic nerve block was on the motor component of the nerve block, while DPN profoundly affects sensory function as well.¹ However, in a previous study using the same model, we obtained comparable prolongations of both motor and sensory block upon sciatic nerve block.¹¹

Ethical considerations

Animal distress was minimized by conducting all invasive procedures such as electrophysiology and nerve block under general anaesthesia, and tissue injury and post-interventional pain were limited by performing nerve blocks percutaneously as described by Kroin and colleagues.¹⁰ However, this approach may result in a less reliable injection site, and intraneural injection is possible. A study directly comparing these two modes of injection found comparable results for both approaches.¹⁰ The same percutaneous approach using thin needle electrodes was chosen for electrophysiological measurements. To avoid repetitive injections of insulin for the animals randomized to the late group, we used subcutaneous implants. To minimize the number of animals used in experiments, we obtained approval to use the heart and the right sciatic nerve of our test animals for other experiments.

Future perspectives

We describe a novel comprehensive model to investigate toxicological and functional consequences of diabetic neuropathy *in vivo*, combining behavioural, electrophysiological, and histopathology methods. Despite lidocaine being the most widely used local anaesthetic for toxicity research, it has been suggested that longer-acting local anaesthetics such as bupivacaine or ropivacaine may be more toxic with respect to neurohistopathology.¹⁰ Investigation of the neurotoxic potential of long-lasting local anaesthetics such as bupivacaine, and the value of adjuvants is therefore required.

Conclusions

Our results suggest increased sensitivity of diabetic nerves to short-acting local anaesthetics without adjuvants *in vivo*, as evidenced by prolonged block duration in a rodent type II DM model with long-standing diabetic neuropathy. This sensitivity appears to increase with the progression of neuropathy. We observed very subtle changes suggestive of nerve injury after nerve block in general, with no correlate in gross behavioural

testing or neurohistopathology, and no specific effect of neuropathy. Our results do not support the hypothesis that neuropathy due to type II DM increases the risk of nerve injury after peripheral nerve block.

Supplementary material

Supplementary material is available at *British Journal of Anaesthesia* online.

Authors' contributions

P.L.: responsible for drafting of research protocol, submission of request for grant funding, submission of animal care and use committee application, performance of experiments, and writing of manuscript. C.V.: assistance in drafting of research protocol (electrophysiology), assistance in submission of animal care and use committee application, assistance in electrophysiological experimental setup, blinded data validation, and assistance writing of manuscript. R.B.: assistance in performing experiments and data analysis, assistance in drafting of research protocol and assistance in writing of manuscript. M.F.S.: assistance in drafting of research protocol, statistical analysis, graphical representation, and assistance in writing of manuscript. W.H.: assistance in data entry and assistance in writing of manuscript. P.G.: assistance in drafting of research protocol (behavioural experiments) and assistance in writing of manuscript. S.B.: assistance in drafting of research protocol and assistance in writing of manuscript. U.G.: assistance in drafting of research protocol (neurohistopathology), blinded neurohistopathological assessment, and assistance in writing of manuscript. I.N.S.: assistance in drafting of research protocol (electrophysiology), electrophysiological data interpretation, and assistance in writing of manuscript. M.W.H.: responsible for drafting of research protocol, submission of request for grant funding, submission of animal care and use committee application, performance of experiments, and writing of manuscript. S.P.: assistance in drafting of research protocol and writing and submission of manuscript.

Acknowledgements

We acknowledge the expert technical assistance of Marian Slaney, BSc, in preparing neurohistopathological samples for analysis, of Shu-Hsien Sheu, MD PhD and Jane Cryan MD, for digitalization of neurohistopathological specimens, and of Susanne van Dieren, PhD, for statistical advice.

Declaration of interest

None declared.

Funding

Supported by the ESRA 2013 Research Grant and the Dutch Association of Anaesthesiology 2012 Young Investigator's Grant (to P.L.), by a M.D.-medical research trainee grant from the Netherlands Organisation for Health Research and Development (ZonMW AGIKO Grant 92003518, to S.P.) and departmental funds.

References

- 1 Said G. Diabetic neuropathy—a review. *Nat Clin Pract Neurol* 2007; **3**: 331–40
- 2 Dhatriya K, Levy N, Kilvert A, et al. NHS diabetes guideline for the perioperative management of the adult patient with diabetes. *Diabet Med* 2012; **29**: 420–33
- 3 Malinzak EB, Gan TJ. Regional anesthesia for vascular access surgery. *Anesth Analg* 2009; **109**: 976–80
- 4 Lirk P, Birmingham B, Hogan Q. Regional anesthesia in patients with preexisting neuropathy. *Int Anesthesiol Clin* 2011; **49**: 144–65
- 5 Lirk P, Rutten MH, Haller I, et al. Management of the patient with diabetic peripheral neuropathy presenting for peripheral regional anesthesia: a European survey and review of literature. *Minerva Anesthesiol* 2013; **79**: 1039–48
- 6 Hebl JR, Kopp SL, Schroeder DR, Horlocker TT. Neurologic complications after neuraxial anesthesia or analgesia in patients with preexisting peripheral sensorimotor neuropathy or diabetic polyneuropathy. *Anesth Analg* 2006; **103**: 1294–9
- 7 Ibinson JW, Mangione MP, Williams BA. Local anesthetics in diabetic rats (and patients): shifting from a known slippery slope toward a potentially better multimodal perineural paradigm? *Reg Anesth Pain Med* 2012; **37**: 574–6
- 8 Sertoz N, Deniz MN, Ayanoglu HO. Relationship between glycosylated hemoglobin level and sciatic nerve block performance in diabetic patients. *Foot Ankle Int* 2013; **34**: 85–90
- 9 Cuvillon P, Reubrecht V, Zoric L, et al. Comparison of subgluteal sciatic nerve block duration in type 2 diabetic and non-diabetic patients. *Br J Anaesth* 2013; **110**: 823–30
- 10 Kroin JS, Buvanendran A, Williams DK, et al. Local anesthetic sciatic nerve block and nerve fiber damage in diabetic rats. *Reg Anesth Pain Med* 2010; **35**: 343–50
- 11 Lirk P, Flatz M, Haller I, et al. In Zucker diabetic fatty rats, subclinical diabetic neuropathy increases in vivo lidocaine block duration but not in vitro neurotoxicity. *Reg Anesth Pain Med* 2012; **37**: 601–6
- 12 Galley HF. Mice, men, and medicine. *Br J Anaesth* 2010; **105**: 396–400
- 13 Brussee V, Guo G, Dong Y, et al. Distal degenerative sensory neuropathy in a long-term type 2 diabetes rat model. *Diabetes* 2008; **57**: 1664–73
- 14 Oh SS, Hayes JM, Sims-Robinson C, Sullivan KA, Feldman EL. The effects of anesthesia on measures of nerve conduction velocity in male C57Bl6/J mice. *Neurosci Lett* 2010; **483**: 127–31
- 15 Price SA, Agthong S, Middlemas AB, Tomlinson DR. Mitogen-activated protein kinase p38 mediates reduced nerve conduction velocity in experimental diabetic neuropathy: interactions with aldose reductase. *Diabetes* 2004; **53**: 1851–6
- 16 Verhamme C, King RH, ten Asbroek AL, et al. Myelin and axon pathology in a long-term study of PMP22-overexpressing mice. *J Neuropathol Exp Neurol* 2011; **70**: 386–98
- 17 Schaumburg HH, Zotova E, Raine CS, Tar M, Arezzo J. The rat caudal nerves: a model for experimental neuropathies. *J Peripher Nerv Syst* 2010; **15**: 128–39
- 18 Thalhammer JG, Vladimirova M, Bershinsky B, Strichartz GR. Neurologic evaluation of the rat during sciatic nerve block with lidocaine. *Anesthesiology* 1995; **82**: 1013–25
- 19 Richardson EP, de Girolami U. *Pathology of the Peripheral Nerve*. Philadelphia: WB Saunders, 1995
- 20 Schmidt RE, Dorsey DA, Beaudet LN, Peterson RG. Analysis of the Zucker Diabetic Fatty (ZDF) type 2 diabetic rat model suggests a neurotrophic role for insulin/IGF-I in diabetic autonomic neuropathy. *Am J Pathol* 2003; **163**: 21–8
- 21 Kalichman MW, Calcutt NA. Local anesthetic-induced conduction block and nerve fiber injury in streptozotocin-diabetic rats. *Anesthesiology* 1992; **77**: 941–7
- 22 Kroin JS, Buvanendran A, Tuman KJ, Kerns JM. Safety of local anesthetics administered intrathecally in diabetic rats. *Pain Med* 2012; **13**: 802–7
- 23 Kroin JS, Buvanendran A, Tuman KJ, Kerns JM. Effect of acute versus continuous glycemic control on duration of local anesthetic sciatic nerve block in diabetic rats. *Reg Anesth Pain Med* 2012; **37**: 595–600
- 24 Oltman CL, Coppey LJ, Gellett JS, Davidson EP, Lund DD, Yorek MA. Progression of vascular and neural dysfunction in sciatic nerves of Zucker diabetic fatty and Zucker rats. *Am J Physiol Endocrinol Metab* 2005; **289**: E113–22
- 25 Etgen GJ, Oldham BA. Profiling of Zucker diabetic fatty rats in their progression to the overt diabetic state. *Metabolism* 2000; **49**: 684–8
- 26 Williams BA, Murinson BB, Grable BR, Orebaugh SL. Future considerations for pharmacologic adjuvants in single-injection peripheral nerve blocks for patients with diabetes mellitus. *Reg Anesth Pain Med* 2009; **34**: 445–57
- 27 Bierhaus A, Fleming T, Stoyanov S, et al. Methylglyoxal modification of Na(v)1.8 facilitates nociceptive neuron firing and causes hyperalgesia in diabetic neuropathy. *Nat Med* 2012; **18**: 1445
- 28 Jain K, Asina S, Yang H, et al. Glucose control and long-term survival in biobreeding/Worcester rats after intraperitoneal implantation of hydrophilic macrobeads containing porcine islets without immunosuppression. *Transplantation* 1999; **68**: 1693–700
- 29 Voelckel WG, Klima G, Krismer AC, et al. Signs of inflammation after sciatic nerve block in pigs. *Anesth Analg* 2005; **101**: 1844–6
- 30 Quinn R. Comparing rat's to human's age: how old is my rat in people years? *Nutrition* 2005; **21**: 775–7
- 31 Lirk P, Haller I, Myers RR, et al. Mitigation of direct neurotoxic effects of lidocaine and amitriptyline by inhibition of p38 mitogen-activated protein kinase in vitro and in vivo. *Anesthesiology* 2006; **104**: 1266–73

Handling editor: H. F. Galley