

Nerve conduction block in diabetic rats using high-intensity focused ultrasound for analgesic applications

Y-F. Lee¹, C-C. Lin², J-S. Cheng¹ and G-S. Chen^{1*}

¹ Institute of Biomedical Engineering and Nanomedicine, National Health Research Institutes, Zhunan, Taiwan

² Department of Neurology, National Cheng Kung University Hospital, Tainan, Taiwan

* Corresponding author. E-mail: gschen@nhri.org.tw

Editor's key points

- The effect of high-intensity focused ultrasound was measured in rats with diabetic neuropathy to assess its potential for pain relief.
- High-intensity focused ultrasound temporarily blocked compound and sensory action potentials and compound muscle action potentials without damaging nerves.
- High-intensity focused ultrasound may have potential for reversibly blocking sensory nerves and providing pain relief.

Background. Nerve conduction block using high-intensity focused ultrasound (HIFU) has been conducted with nerves of mixed fibres in normal animal models. This study tested the feasibility and safety of HIFU for sensory nerve conduction block in diabetic neuropathic nerves to determine its potential for pain relief.

Methods. Diabetes was induced in Sprague–Dawley rats using streptozotocin, and HIFU at 2.68 MHz was used for the block. This study consisted of two sections, *in vitro* and *in vivo*. For the *in vitro* experiments, the entire contiguous sciatic–sural nerves were obtained. Compound action potentials and sensory action potentials were recorded in the sciatic and sural nerves, respectively. For the *in vivo* experiments, compound muscle action potentials (CMAPs) were recorded from the gastrocnemius muscles. All data were expressed as median (range).

Results. The *in vitro* results showed that HIFU temporarily inhibited sensory action potentials of the control and diabetic rat nerves to 33.9 (8.2) and 14.0 (10.7)% of the baseline values, respectively, whereas the compound action potentials were suppressed to 53.6 (8.4) and 76.2 (7.5)% of baseline, respectively. The *in vivo* results showed that HIFU acutely blocked CMAPs to 32.9 (12.6) and 19.9 (10.9)% of baseline in control and diabetic rat nerves, respectively. Measurements of CMAPs and histological examination were used for indirect assessment of the safety of the HIFU technique.

Conclusions. High-intensity focused ultrasound safely and reversibly suppressed nerve conduction in diabetic rat nerves when the stimulation parameters were appropriate. The results suggest that HIFU may have potential to block sensory nerves reversibly and provide peripheral pain relief.

Keywords: diabetic neuropathic nerves; high-intensity focused ultrasound; rat; sensory nerve conduction block

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Nerve conduction block using ultrasound has been studied since the 1960s.¹ *In vitro* experiments using sciatic nerves of the large green frog showed reversible effects on conduction using 10–20 pulses of ultrasound exposure for 0.4–1.0 s.² The increase in temperature induced by ultrasound is one of the mechanisms of inhibition of nerve conduction, and previous experiments have shown that most of these effects occur at 2.7 MHz.^{2–3} In patients with polyneuropathy, the compound muscle action potentials (CMAPs) of the peroneal nerve were reduced after 2 min of low-intensity ultrasound exposure and recovered completely within 5 min.⁴ Recently, the US Federal Drug Administration approved high-intensity focused ultrasound (HIFU) for the non-invasive treatment of uterine fibroid tumours.^{5–6} Furthermore, HIFU has also been applied to suppress nerve conduction and was able to produce a complete and temporary conduction block of bullfrog sciatic nerves

in vitro,⁷ whilst in rats CMAPs in plantar foot muscles were partly and temporarily blocked by HIFU.⁸ However, additional investigations of the effects of HIFU on neuropathic nerves are required. In addition, previous data described the effects on sensory and motor nerves together; thus, the influence of HIFU on sensory nerves alone is uncertain.

Diabetic rat models and the evaluation of neuropathic symptoms are well established.^{9–10} Behavioural tests (e.g. mechanical allodynia and heat hyperalgesia) have been employed to quantify pain in animals.^{11–12} The present study examined the feasibility of blocking nerve conduction, especially the sensory component, using HIFU in neuropathic rats. A streptozotocin (STZ)-induced diabetic rat model was developed, and peripheral neuropathy was confirmed using behavioural tests. Compound action potentials (CAPs) of the sciatic nerve and sensory action potentials (SAPs) of the sural nerve

were analysed *in vitro* before and after HIFU exposure. In addition, the long-term effects of HIFU on the CMAPs of control and neuropathic nerves were examined *in vivo*.

Methods

Animals

The Institutional Animal Care and Use Committee of the National Health Research Institutes in Taiwan approved all animal procedures, and relevant aspects of the ARRIVE guidelines were followed. Diabetes was induced in male adult Sprague–Dawley rats using a single intraperitoneal injection of 50 mg kg⁻¹ STZ (Sigma, St Louis, MO, USA). The body weights and fasting blood-glucose levels of all rats were monitored to confirm diabetes. Rats with fasting blood-glucose levels <150 mg dl⁻¹ 2 weeks after the STZ injections were excluded (Roche Accu-Check Active; Roche, Mannheim, Germany). Peripheral diabetic neuropathy was evaluated using an electronic von Frey device (IITC 2390 series electronic von Frey Anesthesiometer; IITC Life Science, Woodland Hills, CA, USA) and a hot plate (Hot Plate Analgesia Meter; IITC Life Science) to assess mechanical allodynia and heat hyperalgesia, respectively.^{11 12}

In vitro experiments

Rats ($n=12$) were randomly divided into two groups: control rats, which received no STZ injection ($n=6$); and diabetic rats, which had received an STZ injection 4 weeks previously ($n=6$). Rats were anaesthetized by inhalation of 1.75% isoflurane (Panion & BF Biotech Inc., Taoyuan, Taiwan) in oxygen mixtures delivered via a nose cone. The depth of anaesthesia was assessed by the pedal withdrawal response to a nociceptive stimulus. An incision was made to expose the lateral hamstring muscle, and the connective tissues between the medial and lateral hamstring were loosened to expose the sciatic nerve. The sciatic nerve was dissected from its origin to where the sural nerve ends near the ankle. The nerves were then stored in Ringer solution consisting of 146 mM NaCl, 5 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂·6H₂O, 11 mM D-glucose, and 10 mM HEPES buffer (Amresco LLC, Solon, OH, USA). The length of the dissected nerves was approximately 7 cm. After the surgical procedures, the rats were killed under anaesthesia by CO₂ inhalation.

Figure 1 illustrates the experimental configuration. The acrylic nerve platform had three chambers; the central chamber served as the sonication chamber where the nerve was exposed to HIFU, and the left and right chambers were the stimulation and recording chambers, respectively. The electrode wells were filled with Ringer solution to improve electrical contact. The recording chamber had two pairs of electrodes; one pair was placed at the near-knee sciatic nerve, and the other pair was placed at the sural nerve end. Sural nerves are purely sensory nerves, and sciatic nerves are mixed sensory and motor nerves; therefore, the nerve signals from the two sites were SAPs and CAPs, respectively. A needle thermocouple (Omega Engineering, Stamford, CT, USA) was inserted into the

central chamber near the nerve to monitor the temperature of the Ringer solution.

Nerve stimulation and recording were accomplished using a Biopac MP36 acquisition system (Biopac Systems, Inc., Goleta, CA, USA). A stimulus with a pulse width of 0.1 ms and supra-maximal intensity was applied. The CAPs and SAPs were amplified and filtered between 70 Hz and 3 kHz, and the sampling rate was 50 kHz. Details of the probe and HIFU conditions are provided in the online Supplementary material.

In vivo experiments

Additional rats ($n=18$) were randomly divided into the following two groups: control rats, which did not receive STZ ($n=9$); and diabetic rats, which were studied 4 weeks after STZ injection ($n=9$). Rats were anaesthetized using intraperitoneally injected Zoletil 50 (40 mg kg⁻¹, Virbac, Carros, France) and xylazine (10 mg kg⁻¹, Sigma, St. Louis, MO, USA) and placed in ventral recumbency. The depth of anaesthesia was ascertained using the pedal withdrawal response. The sciatic nerves were surgically exposed, and a custom-made nerve fixator was used to ensure the location of the HIFU hot spot on the sciatic nerve. The stimulation site was located at the origin of the sciatic nerve, and CMAPs were recorded from the gastrocnemius muscles. The equipment, stimulus parameters, and sampling rate matched those in the *in vitro* experiments, and both sciatic nerves were treated. The surgical sites were closed using 4–0 chromic catgut sutures (Unik, Taipei, Taiwan). After surgery, the rats were given ibuprofen in the drinking water (Yung Shin Pharm., Taichung, Taiwan) for analgesia.

The duration of HIFU sonication was increased until either the CAP or CMAP signal was suppressed. Data were acquired once every 2 min during the early phase, every 5 min during the middle phase, and every 10 min in the final phase; total recording time was 2 h. Given that an increase in the temperature of the Ringer solution implies that the liquid (rather than the nerve) was heated by HIFU, CAP and SAP data were not analysed if the temperature increased by >2°C in the Ringer solution in the central chamber during sonication. For the *in vivo* experiments, CMAPs were recorded at 7, 14, and 28 days after the initial HIFU exposures.

Histology

Rats were killed by CO₂ inhalation under anaesthesia after 2 h and 7, 14, and 28 days after HIFU exposure. Sciatic nerves were harvested and fixed in 10% formalin, then stained with hematoxylin and eosin by a technician blinded to the treatment of the rats.

Data analyses

All experimental data are expressed as the median (range). The data were analysed using the Kruskal–Wallis test, with *post hoc* paired comparisons.

Results

Rats treated with STZ developed polyuria and lost body weight, with significantly increased fasting blood-glucose levels 4 weeks

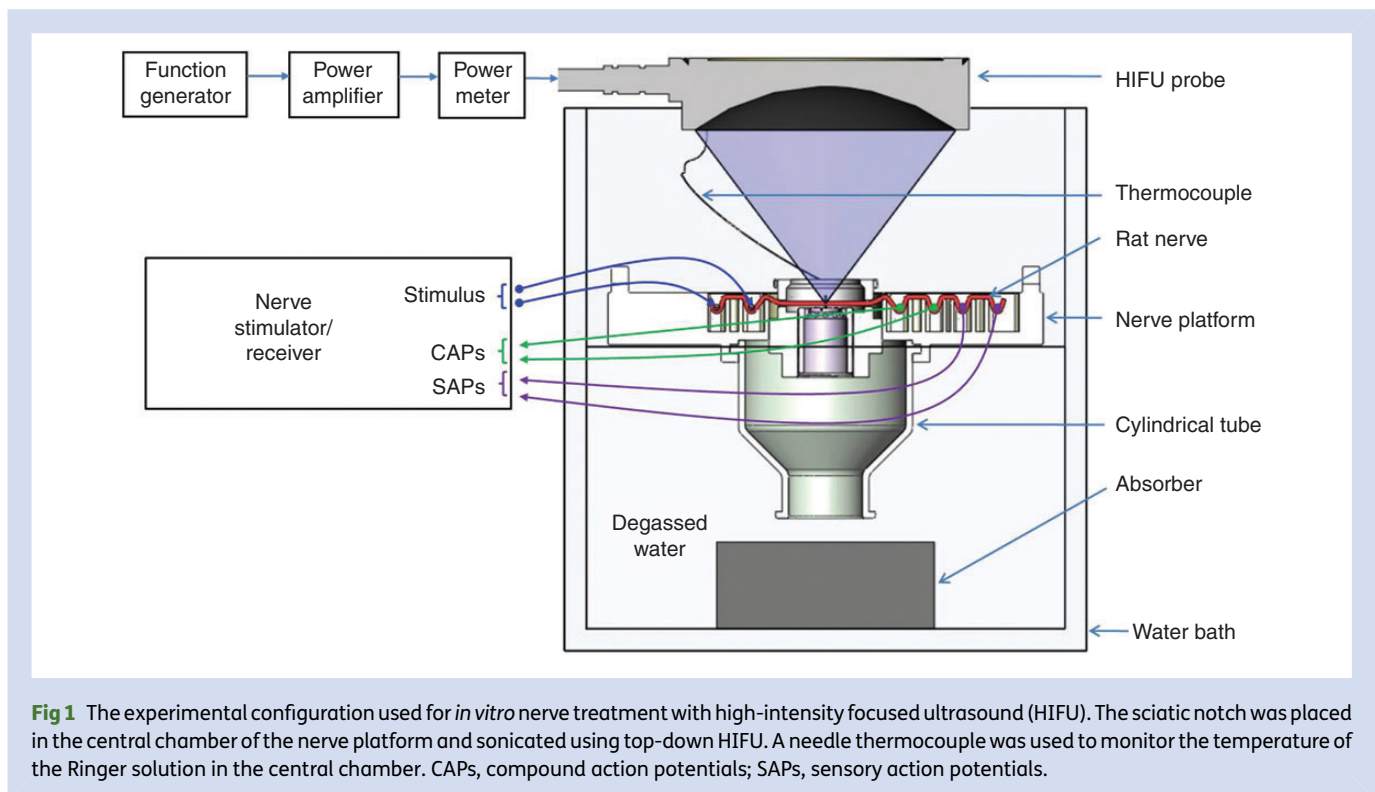


Fig 1 The experimental configuration used for *in vitro* nerve treatment with high-intensity focused ultrasound (HIFU). The sciatic notch was placed in the central chamber of the nerve platform and sonicated using top-down HIFU. A needle thermocouple was used to monitor the temperature of the Ringer solution in the central chamber. CAPs, compound action potentials; SAPs, sensory action potentials.

after STZ injection, indicating diabetes. Diabetic neuropathy manifested as significantly decreased withdrawal latencies and paw withdrawal force. See online Supplementary material for further details.

The CAPs and SAPs of nerves from control rats *in vitro* were temporarily and incompletely suppressed by 4 s of continuous HIFU at an intensity of 2290 W cm^{-2} . The CAPs and SAPs were inhibited by 20% 2 min after HIFU exposure, and maximal suppression was seen (approximately 50%) at 10 min, with 30% suppression at 8 min. Full recovery occurred at 40 and 25 min for CAPs and SAPs, respectively (Fig. 2A). The CAPs and SAPs of nerves from diabetic rats were inhibited by 12.5 and 5.8%, respectively, 2 min after HIFU exposure, and maximal suppression was only 24% at 20 min and 14% at 10 min, respectively, with full recovery occurring at 120 and 80 min, respectively (Fig. 2B).

In the *in vivo* studies, CMAPs in nerves from control rats were suppressed after 7 s HIFU exposure but recovered fully after 8 min (Fig. 3A). The CMAPs at days 7, 14, and 28 were essentially at baseline amplitude levels (Fig. 3B, diamonds). The CMAPs after 12 s of HIFU sonication at the same acoustic intensity decreased at 13 s and did not change significantly after 120 min (Fig. 3A). At 7 days post-HIFU, CMAPs were 69.6 (9.2)% of baseline (Fig. 3B) and were partly recovered by 14 days and fully recovered by 28 days (Fig. 3B). When 18 s sonication was used, CMAPs were abruptly reduced, recovered, and gradually decreased again at 120 min (Fig. 3A). On day 7, CMAPs were approximately 30% of baseline, and they recovered partly by 14 and 28 days (Fig. 3B).

In nerves from diabetic rats *in vivo*, after 3 s of HIFU sonication the CMAPs decreased, but they returned to baseline

after 30 min (Fig. 4A) and were completely recovered by day 7 (Fig. 4B). After 5 s of HIFU sonication at the same intensity, the CMAPs were suppressed at 4 min and recovered by 120 min (Fig. 4A). The CMAPs at 120 min and 7 days were similar and had returned to baseline levels by day 14 (Fig. 4B). After 8 s of HIFU sonication at the same intensity, the CMAPs were reduced at 4 min, but increased at 120 min, and gradually increased to 74% of baseline by day 28 (Fig. 4B, triangles).

Figure 5 shows haematoxylin- and eosin-stained longitudinal sections of the HIFU-treated sciatic nerves. In nerves from control rats, the myelin sheath and axon structure were intact on day 1 after HIFU exposure (2290 W cm^{-2} , 7 s; Fig. 5A), but disruption of the myelin sheath and increased vacuoles or cavities were seen on day 28 after 18 s of HIFU exposure (Fig. 5B). Figure 5C shows a nerve from a diabetic rat on day 1 after HIFU exposure (2810 W cm^{-2} , 3 s). Figure 5D–F are enlarged images of the local regions shown in Figure 5A–C.

Discussion

In vitro, we found that CAPs and SAPs were temporarily and incompletely blocked by HIFU and made a full recovery. Our findings support a previous study showing that the CAPs of normal rat sciatic nerves were partly and temporarily blocked at low exposure (390 W cm^{-2} , 5 s, 5.7 MHz).⁸ The degree of suppression of the HIFU-treated diabetic nerves was less than that of the control nerves, and the duration from suppression to recovery was relatively long.

Only an incomplete block of conduction of the control and diabetic rat nerves was achieved using HIFU, most probably

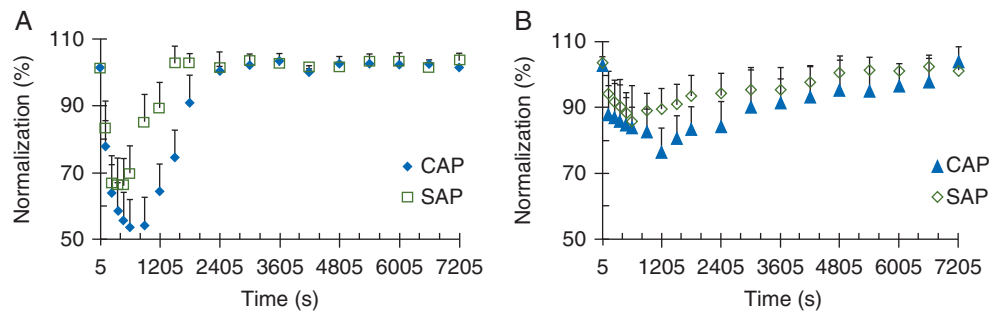


Fig 2 The temporal courses of the CAP and SAP peaks after HIFU exposure of nerves from control (A) and diabetic rats (B) *in vitro*. The incident HIFU parameters for the control and diabetic nerves were 2290 W cm^{-2} for 4 s and 1850 W cm^{-2} for 4 s, respectively. Median values and ranges are shown ($n=12$).

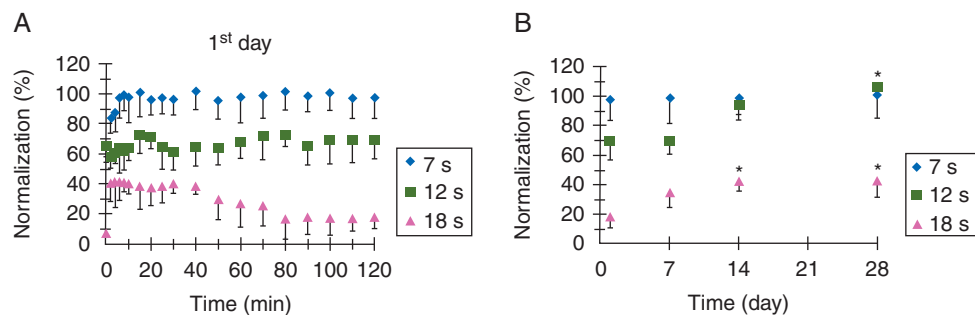


Fig 3 The temporal courses for days 1 (A), 7 (B), 14 (B), and 28 (B) of the compound muscle action potential (CMAP) peaks of control rat nerves *in vivo* after an HIFU exposure of 2290 W cm^{-2} for 7 s (diamonds), 12 s (squares), and 18 s (triangles). $n=6$ for each HIFU parameter. *Significantly different from day 1.

attributable to the different fibre compositions of the nerve. The rat sciatic nerve at the mid-thigh is typically composed of approximately 27 000 axons, 6% of which are myelinated motor axons, 23 and 48% of which are myelinated and unmyelinated sensory axons, respectively, and 23% of which are unmyelinated sympathetic axons.¹⁴ The rat sural nerve generally contains 1100 myelinated and 2800 unmyelinated afferent axons and 1500 unmyelinated sympathetic axons. The differential acoustic properties of these axons can affect the delivered HIFU dose. It has been shown using the brain of cats that acoustic energy can be absorbed primarily by lipid and protein.¹⁵ Given that myelin is primarily fat and absorbs sound very efficiently, myelinated nerves will absorb more of the delivered HIFU than unmyelinated nerves. In addition, thermal conduction can affect the temperature distribution, and it is therefore difficult to block nerve conduction completely without causing damage. The acoustic and thermal properties might explain the variations observed between the nerves of control and diabetic rats. Ultrasound studies have verified that the peripheral nerves of patients with diabetic neuropathy are larger than nerves from non-diabetic patients or diabetic patients without neuropathy.^{16 17} However, there is little information on the ultrasound absorption and thermal conduction

properties of normal nerves compared with diabetic nerves, and differences in one or both of these may explain our findings,^{18 19} because myelin structure, perineurium thickness, connective tissue content, and loss of unmyelinated axons may result in variations in absorption and conduction.^{20–22} In addition, the nerves in the diabetic rats may have a higher threshold and therefore a weaker response to ultrasound.

The fibre composition of the sural nerve is a bimodal distribution of A and C fibres. Large A fibres dominated the SAPs recorded in our experiments. In fact, analgesia is highly related to nociceptor-like C fibres. In the cat, the smallest C fibres in the saphenous nerve are most sensitive to ultrasound, and the largest $A\alpha$ fibres are the most resistant.²³ We hypothesize that the HIFU dose used to suppress the conduction of A fibres might synchronously block the conduction of C fibres because the HIFU-induced temperature increased in C fibres was higher than that in A fibres. As a result, the SAP suppression observed in our experiments suggests that the C fibres were also blocked. The degree of block and the safety of the C fibres treated using HIFU require additional study.

In the present *in vivo* study, the control rat nerves were blocked by $>30\%$ immediately after HIFU treatment and recovered fully after 8 min. Histology demonstrated that the

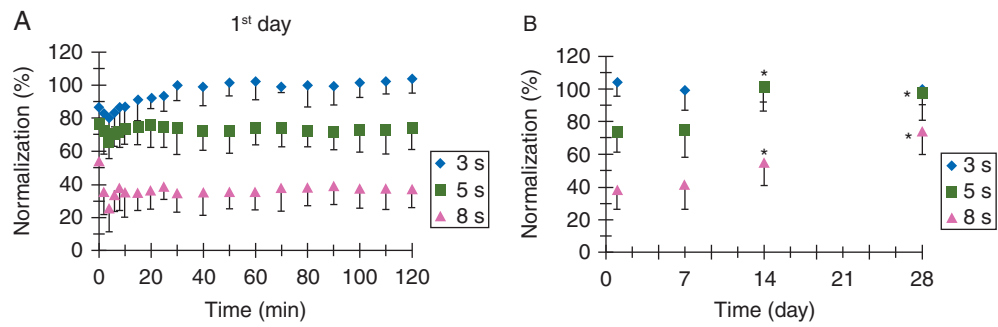


Fig 4 The temporal courses for days 1 (A), 7 (B), 14 (B), and 28 (B) of the CMAP peaks of diabetic rat nerves *in vivo* after an HIFU exposure of 2810 W cm⁻² for 3 (diamonds), 5 (squares), and 8 s (triangles). *n*=6 for each HIFU parameter. *Significantly different from day 1.

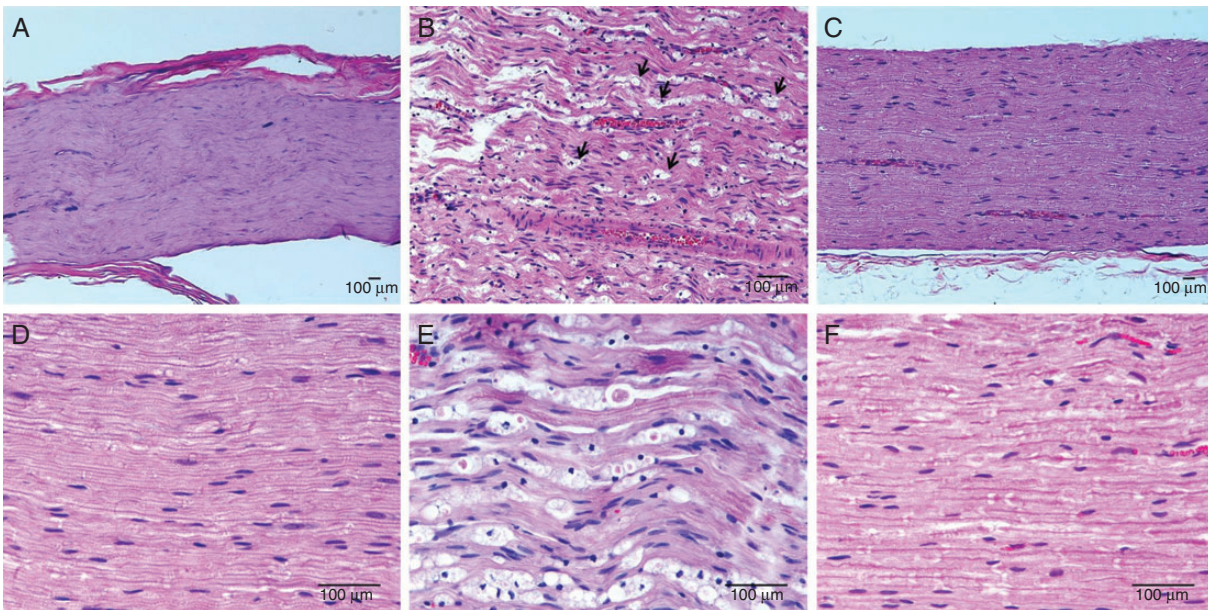


Fig 5 Photomicrographs of haematoxylin- and eosin-stained sections of a representative control rat sciatic nerve on day 1 after HIFU (2290 W cm⁻² for 7 s; A) and on day 28 after HIFU (2290 W cm⁻² for 18 s; B). (C) A diabetic rat nerve on day 1 after HIFU (2810 W cm⁻² for 3 s). (D), (E), and (F) Enlarged images ($\times 400$) of a local region of (A), (B), and (C).

treated control nerves continued to display an organized myelin sheath and axon structure. The follow-up conduction measurements on days 7, 14, and 28 also showed that the CMAPs approximated 100% of baseline. Therefore, an appropriate HIFU dose can suppress conduction without an appreciable adverse effect on normal rat nerves. However, our data suggest that the control rat nerves could be damaged with increased HIFU doses, and histological examination showed that a relatively high HIFU dose was able to disrupt the myelin sheath and increase the number of vacuoles or cavities in the treated nerve. A high HIFU dose might simultaneously induce thermal and non-thermal effects on the nerve;²⁴ high temperatures cause fibre necrosis, whilst cavitation directly bombs the

fibres, both of which reflect granular spaces. Furthermore, the fibres deform into a wave-like shape and may even be disrupted because of the stretch of strong acoustic forces.

Incomplete and temporary conduction block of the nerves from diabetic rats *in vivo* was also observed, with the blocking effect occurring instantly after HIFU treatment. The follow-up CMAPs at baseline and the intact morphology seen on histological examination showed that the temporary conduction block was not harmful at an appropriate HIFU dose. The suppression of nerves from diabetic rats was weak compared with control nerves and concurs with results of the *in vitro* study. However, the degree of suppression *in vivo* was smaller than that seen *in vitro*. This may be explained by intercellular fluid and blood

flow *in vivo* decreasing the absorption of the HIFU and accelerating the dissipation of the HIFU-induced heat. Moreover, blood supply might speed recovery of conduction, so that the recovery time of the nerves *in vivo* is shorter than that seen *in vitro*.

In mild nerve injuries, axonal degeneration is not present and sensory recovery is complete over a period of hours to several days.²⁵ For more severe injuries, the time course of sensory recovery usually takes several months if it occurs at all. In addition, peripheral nerve fibres regenerate more completely when the endoneurial tubes and Schwann cell basal lamina are intact following crushing vs cut injuries.²⁶ As a result, we hypothesize that the HIFU caused mild but reversible nerve injuries seen as decreased CMAPs after HIFU treatment, with return to baseline over time. For more severe nerve injuries, the CMAPs only recovered partly even after 28 days, and histological examination also showed that the fibres were disrupted in certain regions of the nerve.

Previous studies have demonstrated the various parameters that cause thermal injury to neural tissue in different animal models.^{27–30} Based on our results, 2.68 MHz HIFU will damage the rat sciatic nerve if the energy dose is $>275 \text{ J mm}^{-2}$ for the control nerve and $>141 \text{ J mm}^{-2}$ for the diabetic nerve. However, HIFU can block conduction of action potentials in the rat sciatic nerve incompletely and reversibly when an appropriate energy dose is chosen (160 J mm^{-2} for the control nerve and 84 J mm^{-2} for the diabetic nerve), as shown in Table 1s in the online Supplementary material.

In practice and for safety reasons, it is necessary to monitor the temperature of HIFU-treated nerves in real time. We did not measure the temperature of the nerve directly because of the technical difficulty involved. Elsewhere, magnetic resonance imaging thermometry is a useful tool to detect bodily temperature non-invasively.^{31 32} However, the average diameter of the rat sciatic nerve is only 1.0 mm, and the resolution of current magnetic resonance imaging thermometry is insufficient for this application. We found no white lesions in the surrounding muscle tissue after the nerve was heated by HIFU with the dose used in the present study. Although temperature elevation is one of the causes of nerve conduction block,^{2 4 7 33} the mechanism responsible for the reversible inhibition of CAPs remains unclear. The HIFU-induced analgesic effects on normal and diabetic nerves lasted only 8 and 30 min, respectively, based on our *in vivo* investigations, which may limit future clinical use, although repeating the HIFU exposure might extend the analgesia. In addition, the severity of pain may not be proportional to the magnitude of SAP. The pathological decrease of sensory fibres in diabetes mellitus results in hypoaesthesia, but accompanied by allodynia and dysaesthesia. This may reflect the fact that pain in diabetes mellitus is the consequence of peripheral and central interactions. The temporary reduction of SAP by HIFU represents a reduction of peripheral inputs in surviving fibres, similar to the effects of local anaesthetics; therefore, it should be ineffective for blocking the central contribution, but it might be used as a tool to estimate the relative contribution of peripheral and central components.

Blocking nerve conduction in nociceptors will be likely to reduce the experience of pain in acute conditions, but in the

context of chronic pain, where central sensitization plays a role, this approach may not be effective. In addition, a larger HIFU dose would be required to create the effect observed in our study because the tissue in the acoustic path will attenuate the energy and the surrounding tissue will absorb heat.

To the best of our knowledge, the present study is the first to investigate the use of HIFU for nerve conduction blocks in diabetic nerves and lays the foundation for the potential use of HIFU as a pain relief tool in patients with neuropathy. However, further studies are needed, because mixed fibres are more sensitive to suppression than sensory fibres, and thus, some weakness is expected to accompany pain relief. It is important to determine the proper range of parameters so that the side-effects of HIFU are acceptable. In addition, diabetic nerves were more resistant than normal nerves to the blocking effects of HIFU, thereby demanding the use of stronger HIFU treatments to achieve equivalent effects, but this carries a greater risk of nerve damage. Thus, additional studies are needed to investigate the safety of the dose of HIFU required to achieve the desired suppression in diabetic nerves. Although we showed indirect evidence for the safety of the HIFU blocking technique in rat nerves, behavioural pain assessments of rats treated with HIFU must be performed.

We conclude that the reversible conduction block of normal and diabetic rat sciatic nerves can be achieved using HIFU treatment. Our studies demonstrated that a blocking effect occurred immediately post-HIFU treatment and lasted between 10 and 30 min. We also found that provided the HIFU dose was kept within certain parameters, the technique appeared not to cause nerve damage.

Supplementary material

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

Authors' contributions

Y-F.L.: *in vitro* and *in vivo* experiments, data collection, and drafting of the manuscript. C-C.L.: data analysis and interpretation. J-S.C.: *in vitro* and *in vivo* experiments. G-S.C.: conception and design, data analysis and interpretation, manuscript revision.

Declaration of interest

None declared.

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