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● *Original Contribution*

HIGH-INTENSITY FOCUSED ULTRASOUND ATTENUATES NEURAL RESPONSES OF SCIATIC NERVES ISOLATED FROM NORMAL OR NEUROPATHIC RATS

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Abstract—Patients with diabetic neuropathy often have neuropathic pain. The purpose of our work was to investigate the effects of high-intensity focused ultrasound (HIFU) on the conduction block of normal and neuropathic nerves for soothing pain. Adult male Sprague–Dawley rats were used, and diabetes was induced by streptozotocin injection. Diabetic neuropathy was evaluated with animal behavior tests. Sciatic nerves of both control and neuropathic rats were dissected from the starting point of the sciatic nerve to the point where the sural nerve ends near the ankle. The nerves were stored in Ringer's solution. The *in vitro* nerve was placed on a self-developed experimental platform for HIFU exposure. Stimulation and recording of the compound action potentials (CAPs) and sensory action potentials (SAPs) were performed. Control and neuropathic nerves exposed or not exposed to HIFU were submitted to histologic analysis. For the control and neuropathic nerves, suppression of CAPs and SAPs started 2 min post-HIFU treatment. Maximum suppression of SAPs was 34.4 ± 3.2% for the control rats and 11.6 ± 2.0% and 9.8 ± 3.0% for rats 4 wk post-injection and 8 wk post-injection, respectively. Time to full recovery was 25, 70 and 80 min, respectively. Histologic analysis revealed that the nerves in which CAPs and SAPs did not fully recover were damaged thermally or mechanically by HIFU. It is feasible to reversibly block nerves with appropriate HIFU treatment. Diabetic nerves were less suppressed by HIFU and were more vulnerable to permanent damage. (E-mail: gschen@nhri.org.tw) **© 2015 World Federation for Ultrasound in Medicine & Biology.**

Key Words: **Conduction block, High-intensity focused ultrasound, Diabetic neuropathy, Rat, Sciatic nerve.**

INTRODUCTION

There were 25.8 million people in the United States, or 8.3% of the population, who had diabetes in 2010 (Centers for Disease Control [CDC] 2011). Depending on the criteria, diabetic polyneuropathy is estimated to occur in 50% to 90% of individuals who have had diabetes longer than 10 y (Lehtinen et al. 1989; Pirart 1978; Vinik 1999). Pain evoked by diabetic polyneuropathy can degrade quality of life. Unfortunately, the pathogenesis of diabetic polyneuropathy is not well understood, though it is currently believed that some factors lead to reduced Na^+/K^+ ATPase activity and vasoconstriction, thereby reducing endoneurial blood flow and causing nerve hypoxia (Veves and King 2001). Oral medications are typically used for pain relief, and capsaicin cream and lidocaine patches, which are applied topically on the skin, can also help ease pain. Nonetheless, some side effects induced by oral medications still occur. Physical therapies have been proposed for the treatment of painful diabetic polyneuropathy, including acupuncture (Abuaisha et al. 1998), electrical vessel stimulation (Lin et al. 2005), electrical nerve stimulation (Hamza et al. 2000), electrical spinal cord stimulation (Daousi et al. 2005), static magnetic field therapy (Weintraub et al. 2003), low-intensity laser therapy (Zinman et al. 2004) and near-infrared treatment (Leonard et al. 2004). However, the outcome of acupuncture relies greatly on the individual experience of clinicians, and it is as invasive as electrical stimulation. For magnetic field, laser and near-infrared treatments, further studies are needed to prove their effectiveness. In addition, a modality that can instantly soothe the pain is desired by patients with diabetic polyneuropathy because the pain usually occurs at night.

Medical ultrasound has been broadly used for clinical diagnosis; more recently, the U.S. Food and Drug Administration (FDA) approved a high-intensity focused ultrasound (HIFU) system designed specifically for the non-invasive treatment of uterine fibroid tumors (Okada

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et al. 2009; Taran et al. 2009). Nerve conduction inhibition using ultrasound for pain management has been actively investigated for nearly 50 y (Ballantine et al. 1960; Foley et al. 2007). Experiments in surgically exposed cat spinal cords have revealed that reversible effects on reflexes are induced with 3 to 300 bursts of ultrasound exposure, with a duration of 50–300 ms at 2.7 MHz and a repetition rate of once per 0.5–3 s (Young and Henneman 1961b). Ultrasound-induced temperature elevation has been verified as one of the mechanisms underlying nerve conduction block (Lele 1963). *In vivo* experiments in Sprague–Dawley rats indicated that partial and temporary nerve blocks or permanent and complete nerve degeneration could be achieved using HIFU exposures of 390 or 7890 W/cm^2, for 5 s at 5.7 MHz, respectively (Foley et al. 2008). However, the animal subjects used in the above-mentioned experiments were normal, and the nerves were not neuropathic. It is thus necessary to further investigate the effects of HIFU on neuropathic nerves. Conversely, the electrically stimulated compound action potential captured in the above-mentioned experiments is the sum of sensory and motor action potentials, so the influence of HIFU on the sensory nerve is uncertain. In fact, only sensory nerve block is needed for pain relief.

To prove the feasibility of temporarily suppressing neuropathic sensory nerve conduction with HIFU, we developed the diabetic rat model as a neuropathic model and evaluated rat neuropathy in behavior tests. We hypothesized that (i) by choosing appropriate parameters, nerve conduction and pain information transmitted in the nerve could be reversibly and partially blocked by HIFU, and (ii) the blockage was applicable to both normal and neuropathic nerves. An *in vitro* study was performed to simplify the experimental circumstances. We explored the relationship between HIFU and various parameters, including the sensory action potentials (SAPs) and compound action potentials (CAPs) of control and neuropathic nerves.

METHODS

Animal patients

All animal procedures were approved by the Institutional Animal Care and Use Committee of the National Health Research Institutes in Taiwan. Adult, male Sprague–Dawley rats were used in this study. Diabetes was induced in rats by one intraperitoneal injection of streptozotocin (STZ; Sigma, St. Louis, MO, USA) at a dose of 50 mg/kg. To confirm the development of diabetes, weights and fasting blood glucose levels of all rats were monitored using a balance (BJ 8100 D, Precisa, Dietikon, Switzerland) and glucose meter (GC, Roche Accu-Check Active, Mannheim, Germany). Rats with fasting blood glucose levels lower than 150 mg/dL 2 wk after STZ injection were excluded from the experiments. Peripheral diabetic neuropathy (DN) in the rats was evaluated in animal behavior tests, such as the von Frey and hot plate tests (Eddy and Leimbach 1953; Moller et al. 1998). Both sciatic nerves of the control and neuropathic rats were dissected from the starting point of the sciatic nerve to the point where the sural nerve ends near the ankle, as illustrated in Figure 1, and stored in Ringer's solution, which consisted of 146 mM NaC1, 5 mM KC1, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 11 mM D-glucose and 10 mM Hepes buffer (Amresco, Solon, OH, USA). The length of the dissected nerve was approximately 7.0 cm.

Experimental setup

The experimental nerve platform (9.0 × 5.0 × 1.2 cm) made of acrylic had three main chambers. The central chamber (1.8 cm in diameter, 1.2 cm high) served as a sonication chamber, where the nerve was exposed to HIFU. The stimulation chamber (1.3 × 2.0 × 0.9 cm) was divided into three wells by 1-mm-thick partitions, and each partition had a groove 3 mm in diameter for the nerve. A pair of needle electrodes was individually placed in two wells separated by a middle well; the distance between the electrodes was 0.8 cm. The middle well was filled with mineral oil for electrical isolation of the electrodes, and the electrode wells were filled with Ringer's solution for good electrical contact. The recording chamber (3.0 × 2.0 × 0.9 cm) had two pairs of electrodes: one pair was placed at recording site I of the nerve (Fig. 1), and the other pair was at the sural nerve end (recording site II). The nerve signals V_c and V_s illustrated in Figure 2 were thus CAPs and SAPs. Similar to the stimulation chamber, four needle electrodes were partitioned by the middle wells. The electrode and middle wells were filled with Ringer's solution and mineral oil, respectively. A needle

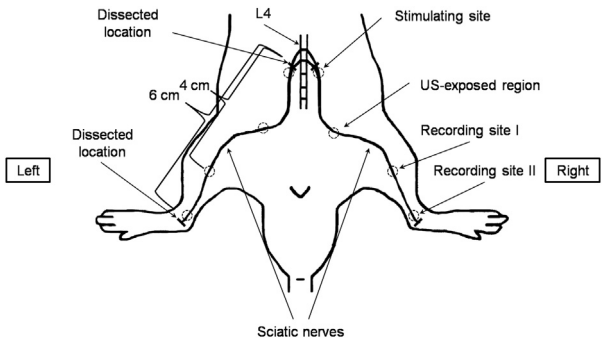

Fig. 1. Dorsal view of the sciatic nerves of a male rat. For the experimental patients, both nerves were dissected from the starting point of the sciatic nerve to the point where the sural nerve ends near the ankle.