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Peripheral Neuritis Trauma in Pigs: A Neuropathic Pain Model

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Abstract: The use of rodents in preclinical studies has contributed greatly to our understanding of the pathophysiology of chronic neuropathic pain. These animal models are limited because of their poor clinical translation. We developed a pig model for chronic pain caused by surgically induced peripheral neuritis trauma (PNT). Seventy-five percent of the animals exhibited mechanical and tactile allodynia, which are indicative of painful neuropathy, by day 28 after surgery. Importantly, the PNT-injured pigs retained their ability to walk or to stand on their injured leg. Messenger RNA analysis of acute inflammatory cytokines calcitonin gene-related peptide and brain-derived neurotrophic factor at the site of injury suggests transient inflammation followed by a persistent high level of neurologic markers. Gabapentin and morphine effectively inhibited hypersensitivity to von Frey filaments and to feather stimuli, and reversed spontaneous pain-related behavior in a dose-related manner. No analgesic effect was detected in PNT-injured pigs after treatment with aprepitant, similar to observations in humans and contrary to observations in rodents. In conclusion, PNT-induced trauma in pigs may comprise a valid preclinical model for the study of the chronification of peripheral nerve injury and for the study of new pain therapies.

***Perspective:** This article presents the characterization of a new peripheral neuritis trauma (PNT) model in pigs. The pig PNT model could help close the translational gap between preclinical and clinical responses and may contribute to improved efficacy or safety of candidate drugs.*

***Key words:** Sciatic nerve trauma, pain chronification, pain behavior, CX3CR1, brain-derived neurotrophic factor.*

Nerve injury in the peripheral or central nervous system can result from various insults, including trauma (either from an accident or from surgery), as well as from inflammatory-medicated or immune-mediated processes. Rodent models of traumatic nerve injury are commonly used for research because of their reproducibility and simplicity.[49] The rat sciatic nerve crush injury model is widely used to assess posttraumatic impairment of motor function.[3,33,47] Peripheral nerve injury methods are also commonly used in the rat. These include, for example, loose ligation of the whole peripheral nerve, known as chronic constriction injury[4]; ligation of a section of a large peripheral nerve or partial sciatic nerve ligation[39]; ligation of the L5 and L6 spinal nerves, also known as spinal nerve ligation[19]; and spared nerve injury, in which 2 of the 3 terminal sciatic branches are cut.[9,35] Rodent models have led to a substantial increase in knowledge of pain mechanisms over the last few decades. However, 1 of their major shortcomings is the frequent failure to predict drug efficacy in humans.[30] The most prominent example of efficacy-related translational failure is with substance P neurokinin 1 (NK-1) antagonist, aprepitant (MK-869).[13] Preclinical evaluation of potential analgesic drugs in higher animal species might contribute to an improvement in translational efficacy.

We developed a peripheral neuritis trauma (PNT) model in an attempt to address the gap between rodent studies and human studies. The pig was chosen for this work because it is considered an excellent model for human disease and exhibits anatomic, physiologic, and neurologic resemblance to humans.[44] Pig skin is known

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for its close physiologic resemblance to human skin, including the function and structure of the nerves as well as the skin innervation.[10,21,44] The skin is the end organ for testing hyperalgesia and allodynia responses, and pigs respond to such tests in a manner that reflects human responsiveness.[5] Given the greater anatomic and neurologic resemblance of pigs to humans, compared with rodents, we hypothesized that the pig PNT model might comprise a useful contribution for understanding pain and for testing new pain therapies. A pig model will also increase the feasibility for evaluating new devices that cannot be evaluated on rodents because of their small size.

The main objective of this work was to characterize the sciatic nerve trauma-induced neuropathic pain model. The benefits and limitations of the model were evaluated based on 3 main categories: 1) behavioral changes, including reflexive hyperalgesia and allodynia measures, operant spontaneous expression, and motor function changes consistent with disturbed pain integration; 2) biomarker and histology analyses of the site of injury and spinal cord; and 3) evaluation of the pharmacologic relevance of this model by assessing the effectiveness of morphine, gabapentin, and aprepitant. The results were anticipated to establish the pig PNT model as a valuable tool for translational efforts that will facilitate the development of neuropathic pain treatments.

Methods

Animals and Housing

Danish Landrace X Large White crossbred pigs from the domestic herd at Lahav Laboratories, Negev, Israel, were used in this study. All procedures and experiments were approved by the institutional animal care and use committee and were designed to minimize the number of animals as well as undue suffering in accordance with the International Association for the Study of Pain.[56] Before the study, all animals were kept under conventional pig production conditions. The animals were housed in open pens (1.4 × 2.4 m) on a 12 h/12 h light/dark cycle 7 days before the start of the study. Feeding occurred 3 times daily using special pig food (Dry Sows; Milobar, Oshrat, Israel). The pigs were provided with opportunities to root and chew for enrichment. Fresh water was provided ad libitum by an automated system.

Habituation

The pigs were habituated to the study protocol for 5 days before surgery, as described previously.[5] The pigs were trained to walk to the preparation room daily during the habituation period to familiarize them with the schedule and the technicians. They were always returned to their original pens with their original penmates. The habituation process was conducted to reduce the pigs' stress level. The temperature in the surgery room was maintained at 19°C (range = 18°C–20°C). The animals were weighed at 6 time points: at the beginning of the acclimatization period, 5 days before surgery (study day –5), on the day of surgery (study day 0) before anesthesia, and after surgery on study days 7, 10, 18, and 28.

Anesthesia and Surgery

Each pig walked freely to the preparation room on the day of surgery. A technician carried the animal in his hands and placed an anesthetic facemask (Akzent Color; Fritz Stephan, Gackenbach, Germany) on the pig's mouth and nose, as described previously.[5] Each animal was anesthetized with a 3% isoflurane/100% oxygen mixture. The technician held the pig until it was relaxed and sleepy. It was shaved and swabbed with 70% ethanol, and was immediately carried to the operating room. The pig was placed in the sternal position on the operating table. The incision area was swabbed with antiseptic liquid polydine solution (Polysept Solution; Rekah Pharmaceutical Industry Ltd., Holon, Israel) and the nonoperated areas were covered with sterile sheets. Blood O_2 saturation was monitored for the duration of anesthesia (Spacelab Medical, Snoqualmie, WA, USA).

Figure 1 (A and B) shows the location of the sciatic nerve and the trauma methodology. An incision of 8 to 10 cm was made through the skin and fascia on the left side of the lower back, toward the caudal end, and approximately 1.5 cm lateral and parallel to the spine line of the pig. The muscles were then retracted and the entire sciatic nerve was exposed. PNT was induced by 3 silk threads (3-0; Assut, Huddersfield, UK), each

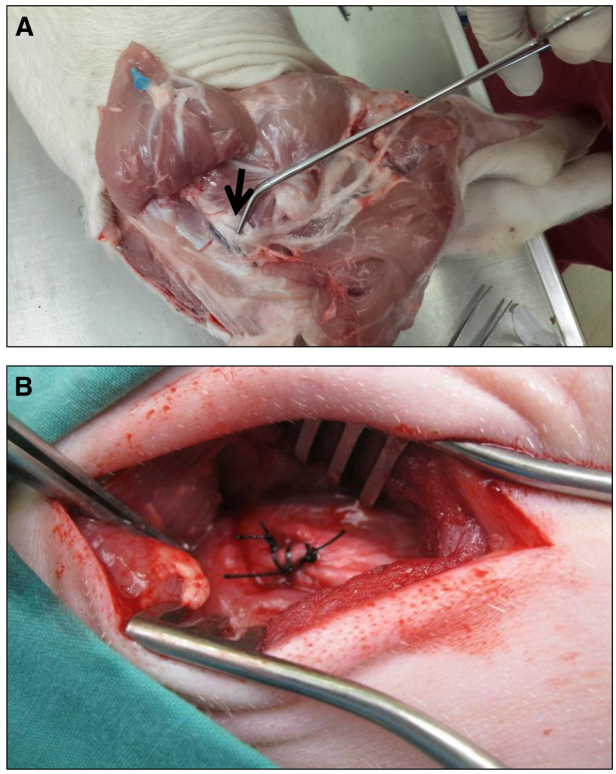

Figure 1. Sciatic nerve trauma methodology. **(A)** Postmortem illustration of injury location. The black arrow shows the area of sciatic nerve injury that innervates the knee and the foot. Note the avoidance of the hind leg innervation. **(B)** Lateral half of the sciatic nerve bundle immediately after PNT, showing 3 loose ligations (1–2 mm apart).