NEW METHODOLOGIES

Ovine Tests of a Novel Spinal Cord Neuromodulator and Dentate Ligament Fixation Method

Katherine N. Gibson-Corley, DVM, PhD,1 Hiroyuki Oya, MD, PhD,2 Oliver Flouty, MD,2 Douglas C. Fredericks, BS,3 Nicholas D. Jeffery, BVSc, PhD,4 George T. Gillies, PhD,5 Matthew A. Howard, III, MD2

1 Department of Pathology, University of Iowa Hospitals and Clinics, Iowa City, Iowa, USA, 2 Department of Neurosurgery, University of Iowa Hospitals and Clinics, Iowa City, Iowa, USA, 3 Department of Orthopaedic Surgery, University of Iowa Hospitals and Clinics, Iowa City, Iowa, USA, 4 Department of Veterinary Clinical Sciences, Iowa State University, Ames, Iowa, USA, 5 Department of Mechanical and Aerospace Engineering, University of Virginia, Charlottesville, Virginia, USA.

ABSTRACT

Background: To improve methods for the treatment of intractable pain, we are developing a novel intradural spinal cord stimulator that could be either attached to the dentate ligaments of the human spinal cord or fitted around the dorsal arc of the cord itself. Purpose: Our goal was to carry out the first in vivo tests of these attachment methods in an ovine model using custom-built devices and instrumentation. For eventual translational studies, we also explored methods of mimicking a human dentate ligament attachment technique in this large animal model. Methods: As a starting point, we investigated details of the gross and histological anatomy of the ovine denticulate ligaments, and compared them with their human counterpart. The gap between the dura and the spinal cord in the sheep is small; hence, the denticulate ligaments are not long enough to accommodate human-scaled attachment clips. Therefore, lateral strips of the spinal-canal dura were fashioned to serve this same device attachment function. Results: This form of dural anchoring was implemented surgically for fixation of a silicone membrane implant that had 12 electrodes, and somatosensory evoked potentials were obtained successfully when stimuli were applied to it. The dorsal arc clamping technique was also implemented. Conclusions: We demonstrated that the dural attachment method is an effective surrogate model for testing the human dentate ligament device fixation technique, and that this mode of fixation was preferable to dorsal arc attachment. The relevant surgical innovations, anatomical findings, and the preliminary electrophysiological data from a pial surface stimulator attached in this way are presented.

Keywords: neuromodulation; spinal cord stimulator; denticulate ligaments; somatosensory evoked potentials; ovine models

INTRODUCTION

Spinal cord stimulation (SCS) is of rapidly growing importance for treating intractable pain [1]. The physiological mechanisms of it are incompletely understood [2] and several factors limit its effectiveness [3–7]. We are introducing a new paradigm SCS device, termed the Iowa-PatchTM (or I-Patch), which is an intradural implant placed directly on the pial surface of the spinal cord [8]. Our initial design called for the I-Patch to be seated circumferentially around the dorsal arc of the spinal cord [9, 10]. Fixation to the denticulate (a.k.a. dentate) ligaments was also considered, as this might allow the device to conform to the pial surface without any inadvertent clamping constriction of the spinal cord tissues. To explore these points, we carried out an in vivo ovine trial. Our specific goals were (1) to investigate the properties of the ovine dentate ligaments, (2) to investigate how dorsal arc clamping compares with dentate ligament anchoring, and (3) to monitor the somatosensory evoked potentials (SSEPs) produced by selective stimulation of the pial surface. The I-Patch prototype tested was a wired version as opposed to a wireless version, as the latter is still under...
development. The novel surgical procedures developed as part of this translational research project are described.

**MATERIALS AND METHODS**

**Ovine Model**

Four adult Suffolk sheep (approximately 60 kg each) were used in this investigation. These animal studies were reviewed and approved by the University of Iowa Animal Care and Use Committee (IACUC# 0902039). These procedures were performed under general anesthesia. Premedication was administered (medetomidine) but no antibiotics were given at any point during the experiments. Each sheep was completely draped, leaving the head and the thoracic segment of the back as well as the left leg exposed. The animal was intubated, artificially ventilated, and anesthetized with isoflurane to induce anesthesia for the surgical intervention and subsequent I-Patch implantation. A total of 30 ml of lidocaine (2%) was injected at the incision sites for the craniectomy, laminectomy/durotomy, and left tibial nerve exposure. Right-sided craniectomy was done and the dura was opened for placement of the subdural grid. This was followed by a multi-level laminectomy and durotomy to expose the dorsal surface of the spinal cord for implantation of the I-Patch (further details are provided later). After induction using isoflurane, anesthesia was then transitioned to propofol IV maintenance at a rate of 0.4 mg/kg/hr through a cannula placed in the jugular vein and isoflurane was discontinued. Corneal reflex was tested periodically to ensure proper depth of anesthesia, and body temperature was maintained within normal limits using a heating pad and warm intravenous saline infusions, as needed. Heart rate, respiratory rate, SpO2, ETCO2, isoflurane depth, invasive blood pressure, and temperature were monitored continuously throughout the experiment. As per the approved protocol and in accordance with the American Veterinary Medical Association (AVMA) 2007 guidelines, all animals were humanely sacrificed with intravenous euthasol (120 mg/kg) until breathing and heart rate stopped. Euthanasia was confirmed via bilateral thoracotomy. Following the procedure, a segment of the spinal cord, including the dentate ligaments, was removed from two of the sheep for histopathologic evaluation. The total duration of surgery and experimentation was approximately 10–15 hrs, with the I-Patch device being in place on the spinal cord for about two-thirds of that period.

**I-Patch Prototypes**

Two substantially different versions of wired I-Patch implants were fabricated by Evergreen Medical Technologies LLC (St. Paul, Minnesota, USA) for testing in the ovine model. The first type, an example of which is shown in the close-up profile view in Figure 1a, was in the form of a 0.5-mm-thick polyetheretherketone (PEEK) band, approximately 6 mm in inner diameter and 21 mm in circumferential length, with an array of 12 electrodes on the inside surface. The individual platinum–iridium electrodes were 0.66 mm in diameter, with small-gauge wires attached to them for connection to the external driving circuitry. As suggested by the device geometry, it was designed to be placed circumferentially around the spinal cord, with the arms then keeping it in place, but with enough radial compliance to allow for soft capture of the underlying tissues. The second type of device is shown in Figure 1b. The substrate material for it was a 0.13-mm-thick silicone sheet, 24 mm in overall length and 6 mm wide, with arms 2 mm wide. This class of device was designed to be completely pliable so that it would...
conform to the surface structure of the spinal cord and then be attached mechanically to either the dentate ligaments or the dura itself by a clipping mechanism. It too had an array of electrodes on the underside, in this case nine of them, 0.3 mm in diameter each, with a lead bundle exiting the dorsal surface side of the device for connection to the external instrumentation.

A range of I-Patch experimental prototypes were designed, constructed, and tested to examine the relative importance of different device features, including substrate thickness and electrode number. Electrode array count and density are key variables that were examined by analyzing differences in brain responses recorded when electrical stimuli were delivered through electrode arrays having different intercontact spacing. The optimum number of electrodes and their geometric spacing for the final-form version of a clinically useful device will be determined during the course of future studies.

**Experimental Procedure**

The first two sheep of the study were implanted with the PEEK band device and the subsequent ones were implanted with the silicone sheet device. Following induction of anesthesia, a right-sided craniectomy was performed and a 3.3 cm x 2.1 cm, 60-contact-point subdural recording grid (Ad-Tech Medical Instruments, Racine, Wisconsin, USA) was placed on the cortical surface. The ability to obtain SSEPs as monitored via this grid was confirmed by electrical stimulation of the contralateral tibial nerve. A multi-level thoracic laminectomy from T7 to T10 was then performed. We noted that the epidural space is very large and allowed for broad lateral exposure of the thecal sac, but that the spinal dura was very thin, in very close proximity to the spinal cord surface, and thus was initially difficult to distinguish from the pial membrane of the spinal cord. However, by using the operating microscope and placement of a durotomy, the dura could be tented for durotomy. Blunt dissection then allowed for the separation of the dura from the underlying spinal cord, which was subsequently kept submerged in warm saline irrigant to maintain normal temperature. With the spinal cord thus exposed, the I-Patch device to be used in that particular animal could then be implanted. A custom applier tool was designed for surgical placement of the circumferential PEEK devices directly on the pial surface of the spinal cord. Full details of its construction and method of use are available elsewhere [11].

The pliable silicone device was secured in place within the spinal canal using Weck™ clips [12], which were surgically applied using a modified Rongeur. The Weck clips are scaled appropriately (1 mm thickness, 5 mm length) for use on the human dentate ligament; however, the sheep dentate ligament is too small to accommodate this clip. To achieve this same lateral attachment function in the sheep, an alternative fixation structure was created by using surgical microscissors to fashion 5-mm-wide strips of lateral spinal canal dura on both sides of the spinal cord. These strips had the same anatomical orientation as the nearby dentate ligaments, and the device under test could thus be attached onto them with proper alignment via the clips. The conceptual features of this experimental arrangement are shown in Figure 2.

During all procedures, operating microscope images of the spinal cord surface were obtained before, during, and after placement of the I-Patch devices. Figure 3a shows the curved PEEK I-Patch device positioned via circumferential fixation onto the spinal cord, while Figure 3b shows the pliable silicone version positioned via attachment of its arms to the lateral dura constructs.

**Stimulation Protocol and Measurement of SSEPs**

While there were certain differences in the specific stimulation protocols applied to each animal, the overall procedure followed the same general approach in all cases. This involved recordings of the SSEPs produced by (1) initial stimulation of the tibial nerve of the left
hind leg, (2) I-Patch stimulation of the dorsal columns of the spinal cord, (3) simultaneous dorsal column and tibial nerve stimulation, and (4) repeat stimulation of the tibial nerve after cessation of I-Patch stimulation. This particular sequence of steps allowed us to make several important observations. The first was to confirm that the subdural grid was placed over the correct cortical area. This was done by observing the distribution of average evoked potentials recorded from the grid. To our advantage, prior knowledge of ovine sensory homunculus served well in placing the subdural grid in the proper position without subsequent need for repositioning. Secondly, this sequence allowed us to generate a reliable baseline for intercomparison of tibial nerve stimulation with SCS. Lastly, the tibial nerve SSEPs could be used to demonstrate how central processing of peripheral stimuli can be modified by SCS.

The full details of these electrophysiological studies will be published separately [13]. In a brief overview, the tibial nerve stimulation was carried out with two needle electrodes delivering monophasic square waves of 0.2-ms pulse width, at constant current. Thereafter, selected electrodes on the I-Patch were driven in the constant-voltage mode, also at monophasic pulse widths of 0.2 ms, by a Tucker-Davis Technologies IZ2 Stimulator (Alachua, Florida, USA). Similar parameters were used for the simultaneous and final tibial nerve-alone stimulation epochs. Some highlights of the data that were obtained are presented next.

**Gross and Histologic Assessment**

After the stimulation recordings were acquired, the animals were sacrificed as per the approved protocol and
Cordotomies were performed to extract the segments of spinal cord on which the I-Patch had been placed. The specimens were immersed in 4% paraformaldehyde for fixation for 48 hr, followed by secondary fixation with 10% neutral buffered formalin for another 48–72 hr. The spinal cord segments were serially cross-sectioned and routinely processed, embedded, further sectioned (4 μm), and stained with hematoxylin and eosin (H&E) for histological examination. Special stains included Masson’s trichrome stain for collagen (e.g., [14, 15]).

RESULTS

The goals of our study were to (1) elucidate the properties of the ovine denticulate ligament, (2) compare the dorsal arc mounting of the I-Patch versus dentate or dentate-like fixation methods of I-Patch fixation on the spinal cord, and (3) demonstrate that well-defined, repeatable SSEPs can be generated by an I-Patch stimulator secured to such spinal canal structures. In what follows, we present the results of each of these components of our study.

Gross and Histologic Description of the Dentate Ligament

In gross anatomical description, and as per Figure 4, the dentate ligaments in sheep in the lumbar spinal cord are approximately 1-mm thick, 0.5-mm long, and are composed of translucent white collagenous tissue. Histologically, they are very similar to their equivalent structures in humans. Figure 5a and c shows that they are extensions of the pia-arachnoid membranes and attach the lateral spinal cord to the overlying dura mater. From inspection, the dentate ligaments appear to be positioned well away from the rootlets that exit via the spinal foramina. The staining in Figure 5b and d further shows that the dense collagenous tissues of which the dentate ligaments are composed are histologically similar to those of the surrounding meninges.

Circumferential Fixation versus Clip Anchoring of the Stimulator Device

A second focus of the study was on the anatomical response of the spinal cord to the two different modes of mounting the I-Patch. Because the device is, by design, intended to be a permanent intradural implant, and because of the delicate, exacting, and costly nature of any procedure that exposes the spinal cord, it is essential that the risk of pial surface inflammation and other such post-operative sequelae be absolutely minimized so as to avoid complications. Toward that end, we histologically examined the spinal cord specimens obtained at cordotomy for evidence of tissue damage as a result of the device implantation procedures. We had noted during the implant surgeries that even when the
Neuromodulator with Dentate Ligament Fixation

special aplier tool [11] was used to carefully situate the circumferentially mounted PEEK devices at their ideal target locations, it was not always possible to avoid a small degree of radial compression of the tissues due to the residual tension within the arms of the device. As per Figure 6, this resulted in the mild, multifocal vascular congestion at points underneath the mounting arms of the PEEK device. However, no such findings were observed in specimens where attachment had been done via clipping of the I-Patch to the dentate surrogates. The complete avoidance of any such issue, no matter how mild, argues strongly for the use of the latter approach in patients.

SSEP Observations during Direct Stimulation of the Pial Surface

Well-defined SSEP waveforms were obtained from the subdural grid on the cortical surface, as per Figure 7a, in each of the experimental animals. Figure 7b shows one example of the data that were recorded. In this case, the stimulus was applied to one electrode of the soft silicone I-Patch affixed to the dentate ligament surrogates, as shown in Figure 3b. The specific electrode was the left most of the middle row, positioned directly over the dorsal midline. The stimulation protocol described previously was used to drive it at an interstimulus interval (ISI) of 1.4 s for 80 presentations, with the anode being located within the subcutaneous tissue. The localized SSEP patterns began to appear at a stimulus threshold of approximately 3 V; the one shown in Figure 7b is a representative pattern obtained at 5 V. Phase reversal lines were easily resolved in the data and the spectro-temporal power changes were distinct in all cases. As a validation check, Figure 7c shows the evoked potential recorded at Channel 35 of the subdural cortical grid, for both I-Patch stimulation and tibial nerve stimulation. A thorough discussion of these electrophysiological findings will appear elsewhere [13].

FIGURE 5 Photomicrographs of the sheep spinal cord in cross-section, highlighting the anatomic location and composition of the dentate ligaments, which are indicated by the arrows in (a, b). H&E-stained sections are shown in (a, c), and Masson’s trichrome-stained sections are shown in (b, d). Scale bar = 1 mm in (a, b) and 100 μm in (c, d). The marked boxes shown in (a, b) contain the magnified images shown in (c, d).
FIGURE 6  (a) Cross-section of the ovine spinal cord, with the slice representing the region immediately below the circumferentially affixed PEEK I-Patch device. The boxes represent zones where subpial (1) and pial-surface (2) close-ups were obtained. Scale bar = 1 mm. (b) In these close-ups, staining revealed points of mild vascular congestion in micro-vessels within the dorsal horn (1) and in surface vessels (2). Scale bar = 100 μm.

**DISCUSSION**

As described previously, the PEEK device, while fully functional, was found to carry a small intrinsic risk of radial compression of the pial tissues and spinal cord. Engineering analysis revealed that the reason for this had to do with the complex nature of the mechanical compliance of devices of the type shown in Figures 1a and 3a. Therefore, we chose to abandon the PEEK devices in favor of the silicone sheet devices attached via the dentate ligament method.

While suturing of a dorsal cord stimulator to the peripheral boundary of a durotomy opening has been described in the literature [16], there are no reported uses of either dural folds or the dentate ligaments as intradural anchoring points for such devices. However, the programmed stimulation of the dorsal column fibers by selective activation of the silicone sheet’s electrodes repeatedly produced well-defined SSEP patterns, thus indicating that stable conformation of the pliable device body onto the pial surface of the spinal cord had been achieved via this anchoring method. Because the human dentate ligaments are a factor of 3–5 times wider and broader than the ovine ones, they will constitute a larger structural member to which the fixation clip can be secured,
FIGURE 7  (a) Photograph of the 60-contact-point subdural recording grid in place on the cortical surface of the brain. (b) A representative pattern of the somatosensory evoked potentials resulting from a 5 V direct stimulation of the sheep spinal cord along the dorsal midline using the pliable silicone device, with lateral attachment arms affixed to the dentate ligament surrogates. (c) Comparison of the evoked potentials recorded on Channel 35 of the subdural cortical grid for both I-Patch stimulation (red trace) and tibial nerve stimulation (blue trace).
and we conclude that the intradural stimulator fixation method explored in our study might be useful in patients.

ACKNOWLEDGMENTS

The prototype I-Patch devices were fabricated by Evergreen Medical Technologies LLC, and we thank R. Shurig, S. Scott and R. Nelson for their efforts. We also thank our colleagues in the U.Iowa/U.Virginia I-Patch collaboration for several useful discussions. The work at the University of Iowa was funded in part by the GIVF Seed Funds program, and that at the University of Virginia was funded in part by the Kopf Family Foundation, Inc.

Declaration of interest: M. A. Howard and G. T. Gillies may receive patent royalties from any commercial licensing of the Iowa-Patch™ intellectual properties that might be negotiated by their respective institutions.

Author contribution statement: Authors Gibson-Corley and Oya contributed equally to the work as co-first-authors. They and the remaining authors all contributed appropriately to the experimental design, the study itself, the data analysis, and the preparation of the manuscript.

REFERENCES