Journal of Neuroscience Methods 200 (2011) 36–40

Contents lists available at ScienceDirect

Journal of Neuroscience Methods

journal homepage: www.elsevier.com/locate/jneumeth

Catheterization of the thoracic spinal subarachnoid space in mice

Y.Y. Poon[a,b], Alice Y.W. Chang[b], S.F. Ko[c], Samuel H.H. Chan[b,*]

[a] Department of Anesthesiology, Kaohsiung Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Kaohsiung, Taiwan, ROC
[b] Center for Translational Research in Biomedical Sciences, Kaohsiung Chang Gung Memorial Hospital, Kaohsiung 83301, Taiwan, ROC
[c] Department of Radiology, Kaohsiung Chang Gung Memorial Hospital and Chang Gung University College of Medicine, Kaohsiung, Taiwan, ROC

ARTICLE INFO

Article history:
Received 31 March 2011
Received in revised form 15 May 2011
Accepted 14 June 2011

Keywords:
Catheterization
Thoracic spinal cord
Subarachnoid space
Mice

ABSTRACT

The availability of genetically manipulated mice offers a golden opportunity for the study of the contribution of the genome to diseases. Because of the technical difficulty in performing spinal subarachnoid catheterization in mice, this opportunity has hitherto been less harnessed in investigations on the role of the spinal cord in the physiological or pathological processes. Even less explored are spinal mechanisms that underlie cardiovascular regulation since subarachnoid catheterization of the mouse thoracic spinal cord, where preganglionic sympathetic neurons governing vasomotor tone are located posts the highest challenge because of the restricted operating area. We report a procedure for subarachnoid catheterization of the thoracic spinal cord in mice that did not require laminectomy or drilling of the lamina proper, and compared the suitability of two candidate catheters, polyethylene PE-5 catheter (0.51 mm, OD) and polyurethane PU-10 catheter (0.25 mm, OD). Whereas all implanted mice resumed normal feeding one day after surgery and were devoid of bladder dysfunction or self-mutilation, the smaller and softer PU-10 catheter compared favorably because of lower post-operative mortality rate and no unilateral lower limb paresis.

1. Introduction

The availability of genetically manipulated mice offers a golden opportunity to study the contribution of the genome to the initiation and progression of many diseases (Gama Sosa et al., 2010). This opportunity has hitherto been less harnessed in investigations on contribution of the spinal cord to the physiological or pathological processes. One research area that has received more attention is delivery of test agents to the lumbar spinal cord in the study of pain and analgesia (Fairbanks, 2003). For example, intradural injection via direct lumbar puncture in mice, first reported by Hylden and Wilcox (1980), is still in use in recent investigations on pain mechanisms (Cope et al., 2010; Vulchanova et al., 2010; Asiedu et al., 2011). Subarachnoid administration of drugs to the lumbar cord of mice via chronic catheterization (Wu et al., 2004) has also been reported for the study of nociception. Because preganglionic sympathetic neurons governing vasomotor tone are located in the thoracic spinal cord, it is best suited for investigation of spinal mechanisms that underlie cardiovascular regulation. However, thoracic spinal subarachnoid catheterization in mice for sampling of cerebrospinal fluid (CSF) and administration of drugs posts the highest challenge because of the most restricted operating area.

We report a method for thoracic spinal subarachnoid catheterization in mice. We also compared the suitability of two candidate catheters, polyethylene PE-5 catheter (0.51 mm, OD) and polyurethane PU-10 catheter (0.25 mm, OD). Whereas all implanted mice resumed normal feeding one day after surgery and were devoid of bladder dysfunction or self-mutilation, the smaller and softer PU-10 catheter compared favorably because of lower post-operative mortality rate and less unilateral lower limb paresis.

2. Materials and methods

2.1. Animals

All experimental procedures conformed to the guidelines approved by our institutional animal care and use committee. Adult male ICR mice (30–35 g) purchased from the Experimental Animal Center of the National Science Council, Taiwan, Republic of China, were used. They were housed under temperature control (24–25 °C) and 12-h light–dark cycle. Standard laboratory rodent chow and tap water were available *ad libitum*.

* Corresponding author. Tel.: +886 7 7317123x8599; fax: +886 7 7317123x8569.
E-mail addresses: shhchan@adm.cgmh.org.tw, shhchan888@gmail.com (S.H.H. Chan).

0165-0270/$ – see front matter © 2011 Elsevier B.V. All rights reserved.
doi:10.1016/j.jneumeth.2011.06.010

2.2. Preparation of catheters

Two types of catheters made of different materials and sizes, PU-10 and PE-5 (Strategic Applications Inc., Libertyville, IL, USA) were used. For both types, a color mark was made at one end of a 5 cm-long catheter, 0.5 cm from the tip. In addition, 0.1 cm of the other end of the PU-10 catheter was inserted into a 2.5 cm-long PE-5 catheter that acts as a conduit, and the junction was sealed with epoxy glue. The catheters were sterilized in ethylene gas before use.

2.3. Subarachnoid catheterization

Sixty mice were anesthetized with pentobarbital sodium (50 mg/kg, ip). A rectangular area of the skin (2.5 cm × 4.5 cm) overlying the T6 to L5 vertebrae was shaved and sterilized with povidone iodine. A midline incision was made (Fig. 1a), and the left paravertebral muscle attached to the T11 to L1 vertebrae was reflected from the spinous processes (Fig. 1b). Under the guidance of a surgical microscope, part of the supraspinous ligament and the left interspinous ligament between T12 and T13 vertebrae were removed. The interspinous space between T12 and T13 was then exposed, and the underlying epidural fat was carefully removed to reveal the dura (Fig. 1c). After application of a small drop of 4% lidocaine to the exposed dura, and under higher magnification of the surgical microscope, a slit was made by traversing the surface of the dura with the tip of a 30-gauge needle, resulting in leakage of clear CSF. The marked end of the PU-10 or PE-5 catheter was pinched by a pair of forceps for insertion through the slit. The catheter was advanced as tangentially as possible into the subarachnoid space until the color mark (Fig. 1d) reached the slit, indicating that the tip of the catheter was lodged at the T12 spinal cord. A small drop of tissue glue (Histoacryl; B. Braun, Tuttingen, Germany) was then applied over the entry point of the dura to prevent inadvertent slippage of the catheter from the subarachnoid space and leakage of CSF. A 33-gauge needle connected to a 1-mL Hamilton (Reno, NV, USA) microsyringe filled with sterile artificial CSF (aCSF) was inserted into the free end of the PE-5 catheter or the PE-5 conduit connected to the PU-10 catheter. After flushing with 3–5 μL of aCSF, the exterior end of the catheter was sealed by heat. The catheter was further secured on the fascia of the paravertebral muscle with suture, and the sealed end was buried under the skin. The wound was closed in layers, and sodium penicillin (1000 IU; YF Chemical Corporation, Taipei, Taiwan) was given intramuscularly to prevent postoperative infection. Animals were returned to the animal room for postoperative recovery in individual cages.

2.4. Criteria for successful subarachnoid catheterization

We defined successful subarachnoid catheterization by four criteria. First, CSF could be withdrawn on slight aspiration by the microsyringe after the catheter was secured by tissue glue during surgery. Second, animals resumed normal feeding during the interim between surgery and experiment, which was normally 7 days. Third, there was a lack of motor deficits, leakage of CSF at the wound, bladder dysfunction (defined as urinary incontinency or urinary retention) or self-mutilation after operation. Fourth, Indian ink injected at the end of the experiment exhibited only subarachnoid distribution.

2.5. Myelographic evaluation of the thoracic spinal subarachnoid catheterization

Six mice with implanted subarachnoid catheter were anesthetized with pentobarbital sodium (50 mg/kg, ip). The catheter was retrieved through a small incision, and was flushed with 3–5 μL of aCSF. Three successive intrathecal administrations of a contrast medium (Opaque; GE Health, Cork, Ireland), given 5 min apart and at 3, 6 or 9 μL, were delivered at a rate of 5 μL/min by a microdialysis pump (CMA/102; CMA Microdialysis, Stockholm, Sweden). Myelographic examination was performed using a digital mammographic set (Selenia; Hologic Inc., Danbury, CT, USA). Because of the size of a mouse, the employment of high penetrating X-ray power and low exposure time would decrease the contrast and blackness of the film. As such, a combination of low kilovoltage, high milliampere seconds and high magnified field was used to optimize the quality of the images. Radiographs were taken in the prone position 2 min

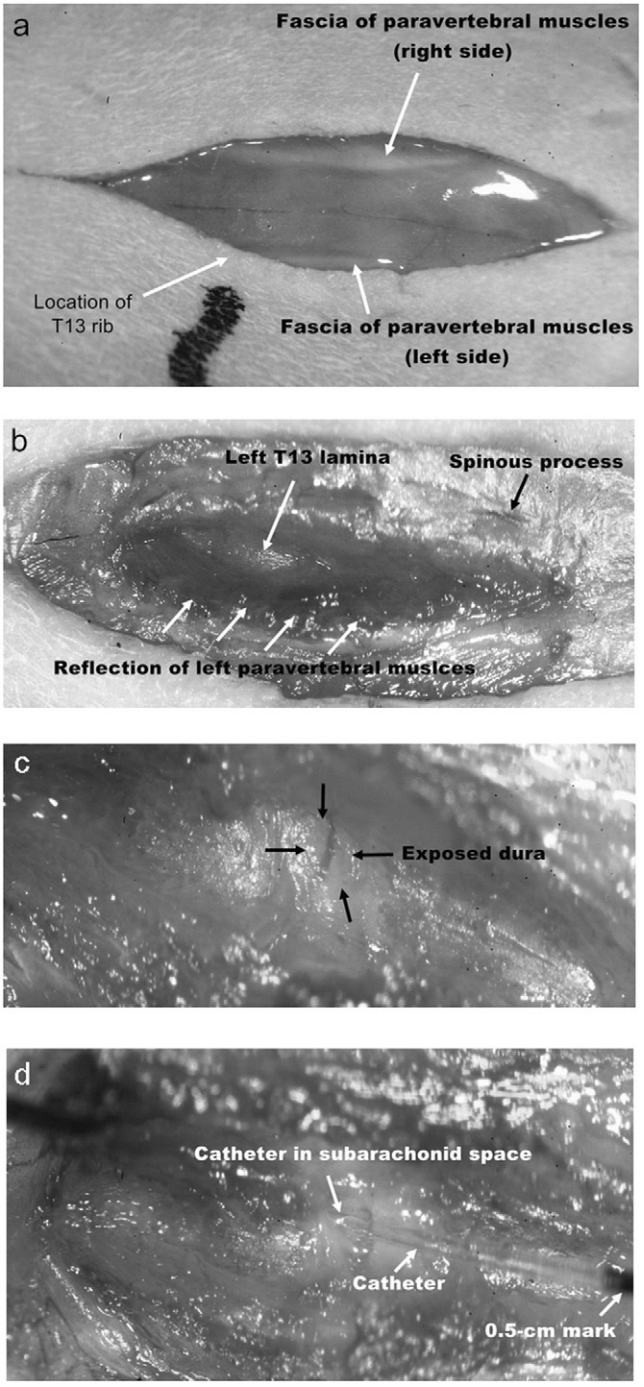

Fig. 1. Photographic illustration of procedures for catheterization of the thoracic spinal subarachnoid space in mice. (a) Skin incision over T10 and L2. (b) Reflection of left paravertebral muscle. (c) Exposure of intervertebral space of T12 and T13. (d) Insertion of catheter in subarachnoid space via the slit on dura.