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Research article

Development of a simple, rapid, and robust intrathecal catheterization method in the rat

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HIGHLIGHTS

- A simple, rapid and robust method for the intrathecal catheterization of rats was developed.
- The method uses a lumbar spinal approach minimizing insertion of catheter material into the thecal space.
- Readily available materials and simply created custom guide cannulas are used in the method.
- This method has been taught to many individuals and has been used for the placement of thousands of intrathecal catheters in rats.
- Little to no adverse effects of the placement of the intrathecal catheters have been observed with this method.

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ABSTRACT

Background: The blood brain barrier (BBB) is an impediment to the development of large and highly charged molecules as therapeutics for diseases and injuries of the central nervous system (CNS). Antisense oligonucleotides (ASOs) are large (6000–8000 MW) and highly charged and therefore do not cross the BBB. A method of circumventing the blood brain barrier to test ASOs, and other non-BBB penetrant molecules, as CNS therapeutics is the direct administration of these molecules to the CNS tissue or cerebral spinal fluid.

New method: We developed a rapid, simple and robust method for the intrathecal catheterization of rats to test putatively therapeutic antisense oligonucleotides. This method utilizes 23-gauge needles, simply constructed ½ in. long 19-gauge guide cannulas and 8 cm long plastic PE-10 sized catheters.

Comparison with existing methods: Unlike the cisterna magna approach, this method uses a lumbar approach for intrathecal catheterization with the catheter residing entirely in the cauda equina space minimizing spinal cord compression. Readily available materials and only a few specialized pieces of equipment, which are easily manufactured, are used for this intrathecal catheterization method.

Conclusions: This method is easy to learn and has been taught to multiple in house surgeons, collaborators and contract laboratories. Greater than 90% catheterization success is routinely achieved with this method and as many as 100 catheters can be placed and test substance administered in one 6-h period. This method has allowed the pre-clinical testing of hundreds of ASOs as therapeutics for CNS indications.

1. Introduction

Ionis Pharmaceuticals Inc. develops antisense oligonucleotides (ASOs) as therapeutic drugs. ASOs do not cross the blood brain barrier (BBB) because of their large size (6000–8000 MW) and net charge. In order to use ASOs as neurological therapeutics, they must therefore be dosed directly into the central nervous system, either intraparenchymally, or into the cerebral spinal fluid (CSF). In human clinical programs, we administer subarachnoid intrathecal bolus doses of ASO to the CSF via lumbar puncture of the cauda equina space. In order to model this route of administration in pre-clinical studies, we have developed a method in rats that closely mimics our clinical dosing paradigm. Initially, lumbar puncture in rats was attempted, but the success rate in delivering ASOs using this method was low and dosing solution often leaked out of the hole made in the thecal sack. We then attempted to adopt the atlanto-occipital intrathecal catheterization method pioneered in

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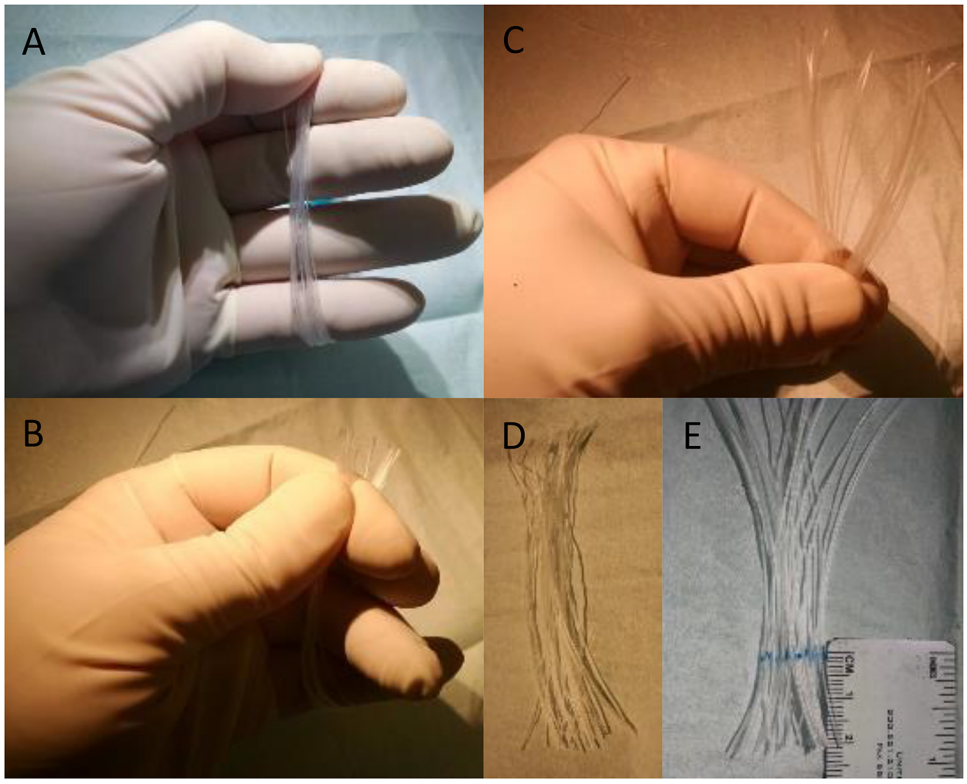

Fig. 1. Photographs of the catheter making process whereby PE-10 tubing is wrapped around the fingers of the hand (A) and then cut at the top (B) and bottom (C) to make two approximately 8 cm long catheters for every wrap of the fingers (D). The catheter is then marked with alcohol resistant ink 2 cm from one end (E).

the laboratory of Tony Yaksh (Yaksh and Rudy, 1976; Malkmus and Yaksh, 2004), but we found that the method was not very rapid, required specialized equipment and, in our hands, there was a high incidence of spinal cord damage. After reading about the lumbar approach for intrathecal catheterization (Martin et al., 1984; Storkson et al., 1996; Jasmin and Ohara, 2001) we settled on this route for dosing rats. This approach allows a small length of catheter to be threaded into the spine as opposed to the longer catheter used in the atlanto-occipital approach, reducing the potential for spinal cord compression effects. We simplified the published method by using unmodified tubing for catheters and a minimally invasive surgical approach with catheterization directed using a needle and guide cannula. This resulted in a straightforward and rapid method in which the catheter can be heat sealed and left in place to block the hole made in the thecal sack reducing leakage of the dosing solution. Our overarching goal was to develop a method that would not require special equipment and would use minimally modified materials that were readily available. The resulting method is inexpensive, has been readily transferred to many academic and contract research laboratories across the world, when surgeries are multiplexed requires only 3–4 min to dose a single rat, and produces a greater than 90% success rate of ASO delivery to the CNS.

2. Methods

2.1. Overall approach

The goal of developing this method was to have a simple, precise, and high throughput way of dosing ASOs into the intrathecal space of rats. The first step was to simplify catheter assembly. Other published methods of intrathecal catheterization have relatively complex catheter designs fusing different sizes of tubing or stretching the catheters to modify their diameter (Yaksh and Rudy, 1976; Storkson et al., 1996). We decided to utilize unmodified PE-10 polyethylene tubing (BD Intramedic tubing 427401, ID 0.011" × OD 0.024"). We adopted 8 cm long catheters, as this is the approximate distance across the fingers of the hand. We make the catheters by wrapping the PE-10 tubing around the fingers of the hand and cutting the tubing with sharp scissors along the top and bottom to create two approximately 8 cm long catheters for each wrap of the hand (Fig. 1).

We decided early in the method development to place the internal end of the catheter completely in the cauda equina space to minimize spinal cord compression and to mimic our clinical dosing paradigm. Several different sized rats were euthanized and their lumbar spines dissected so that the distance from the junction of lumbar vertebrae L5 and L6 (insertion location of the catheter) to the caudal end of the spinal cord could be measured (Fig. 2). Adult

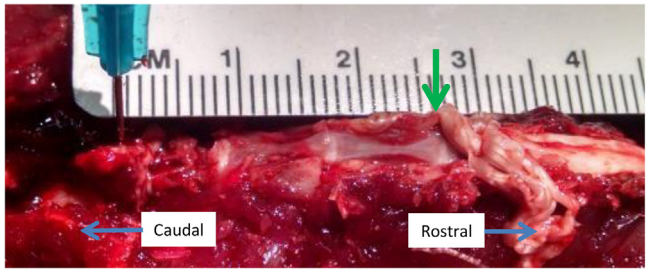

Fig. 2. Photograph of the dissection of the cauda equina space of an adult rat. A 23 gauge × 1" long needle has been placed in the spine at the L5–L6 intervertebral space where the catheter is inserted. The spinal nerves of the cauda equina have been pulled away to reveal the spine. The caudal end of the spinal cord can be seen just to the right of the green arrow that designates its location in the cauda equina. The ruler demonstrates that there is at least 2 cm of space from the insertion point of the catheter to the caudal end of the spinal cord. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)