Optimization of Intrathecal Administration in the Rat

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1 ABSTRACT

Over the past few years, there has been increased interest in using the intrathecal route of administration for targeted delivery of molecules to the central nervous system (CNS). This approach is more challenging in rodents than in large animals due to their small size and associated dose volume limitations. Refinement of the surgical techniques, including improving the rate of adequate catheter placement and reduction in the stress/pain related to the puncture of the access port is imperative. Our laboratory has modified its surgical procedure by introducing the use of Instech PinPorts™ for delivery of agents in Sprague-Dawley rats. PinPorts™ are designed to allow the exteriorization of the catheter while maintaining a closed system, therefore avoiding puncturing through the skin for each test material administration and reducing the pain and stress caused to the animal during manipulations. In addition, these access ports allow reduction of the overall volume to be injected due to its minimal dead volume.

After surgical placement of the catheter and port, the animals were kept for an observation period of up to 14 weeks. Clinical signs, body weights and clinical pathology parameters were monitored and patency of the catheter system was also confirmed by injecting 0.02 mL of 0.9% Sodium Chloride for Injection, USP at least once weekly. The animals were then euthanized and the placement of the catheter was confirmed. The rate of success for the placement within the intrathecal space was 88% and patency was maintained for all animals throughout the observation period. The bodyweight of the animals was unaffected over the observation period and there were no adverse clinical signs noted. The clinical pathology profiles were also within historical data at our laboratory. Overall, this demonstrates that the use of Pinports™ was associated with successful intrathecal administration in rats.

2 MATERIALS AND METHODS

Forty one male Sprague-Dawley Crl:CD(SD) rats were obtained from Charles River Canada Inc. At time of implantation, rats were approximately 9 weeks old and weighed between 202 and 285 g. Animals were housed individually in polycarbonate bins equipped with an automatic watering valve and appropriate bedding material. Temperature and humidity were maintained between 19 mand 25°C and 30 and 70%, respectively, and animals were fed a standard rodent pelleted diet ad libitum.

All animals had a polyurethane catheter inserted through an incision in the atlanto-occipital membrane and passed caudally to the lumbar level. The distal part of the catheter was exteriorized and connected to a Pinport™ access port (Instech). Animals received a standard protocol of anesthesia and antibiotics. Patency checks of the catheter system were performed once weekly following surgeries using 0.02 mL of saline continuing for up to 14 weeks. The Pinport™ was wiped with alcohol prior to any injection.

Morbidity/mortality checks were performed at least twice daily. Detailed examinations and body weight measurements were performed once prior to implantation and weekly thereafter. Functional observation battery (FOB) evaluation was conducted 1 week following surgery on a portion of the animals. Blood was collected for hematology, biochemistry and coagulation investigations at least 20 days after implantation. Fourteen (14) weeks after surgery, animals were euthanized and were subjected to a partial necropsy. Catheter localization was confirmed and the carcasses were discarded.

Figure 1: Injection using a PinPort™

Image taken from Instech Solomon website

3 RESULTS

Animals recovered well from surgeries. Patency was maintained in all animals over the entire observation period. In 4 animals, the Pinport ™ was found to be detached (during week 4, 5, 7 and 9). In 2 cases a repair was attempted and was successful.

There were no abnormal clinical signs recorded. Incidental observations such as scabbing of the skin, thinning of the fur or skin lesion were noted in individual animal at an incidence considered normal for this stain and age of animals. The home cage, arena, handling and on surface assessments as part of the qualitative FOB showed no abnormal findings. Results of the quantitative evaluations (rearing events, forelimb and hindlimb grip strength and hindlimb splay) were also within normal range (Figure 2). Animals showed normal body weight gain over the period of observation (Figure 3).

White blood cell, lymphocyte, neutrophil and red blood cell counts were within historical range for most animals (Figures 4 to 7). Excursions from the historical ranges were still considered to be within normal biological variation based on the minimal extent of excursions.

Catheter placement was confirmed by macroscopic examination at necropsy. In 36 animals out of 41 (88%), the catheter was correctly placed in the intrathecal space. In 4 animals, the catheter was found to be in the spinal cord parenchyma (10%), and in 1 animal the catheter could not be located. These findings are in line with previously published data (Butt, M., Toxicol Pathol 2011 39:213).

3 RESULTS

Figure 2: Quantitative FOB

	Rearing	Grip strength Forelimb (g)	Grip strength Hindlimb (g)	Hindlimb splay (cm)
Minimum	2	546	313	6.0
Maximum	17	1044	493	12.7
Mean	**7.8**	**856**	**414**	**8.9**

Figure 3: Body Weights

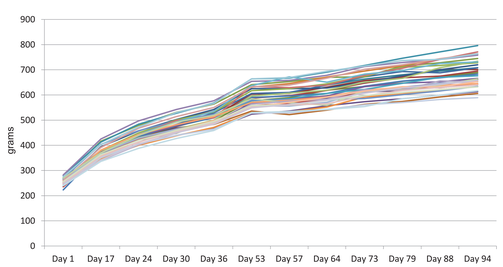


Figure 4: White Blood Cell Count

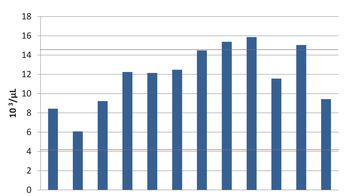


Figure 5: Lymphocyte Count

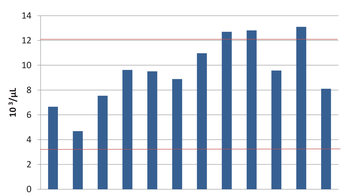


Figure 6: Neutrophil Count

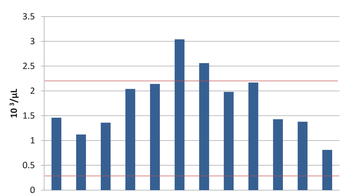


Figure 7: Lymphocyte Count

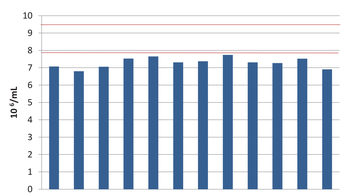
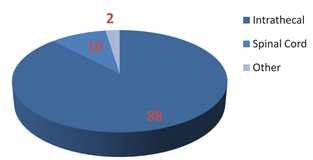


Figure 8: Catheter Placement



4 CONCLUSIONS

Intrathecal administration was successfully conducted in our laboratory using the Pinport™ system from Instech Solomon instead of a traditional subcutaneous access port. Our data show that in addition to reducing the stress to the animal and reducing the dead volume of the catheter system, the Pinport™ was associated with maintenance of patency over at least 14 weeks. Standard toxicology parameters were not impacted by the implantation and macroscopic findings were minimal and not adverse. Finally, optimization of the implantation technique allowed accurate placement of the catheter in the intrathecal space in 88% of the animals, showing that the use of PinPort ™ presents a reliable option for repeated intrathecal injections.

For studies with less frequent dose administration, direct injection in the intrathecal space under anesthesia can also be performed. This approach necessicitate incision of the skin in the lumbar area to allow good visualization of the vertebraes. A spinal needle (25G) or a hypodermal needle (30G) is slowly inserted until CSF appear in the hub. A syringe is then connected and the test material is injected slowly. The needle is then retracted and the skin is closed with suture. Using the same approach, CSF samples (up to 30 µL) can also be collected.

5 Acknowledgements

The authors would like to thank Chantal Bossé as well as the technical and scientific staff at Charles River Montreal for their contribution.