

# Intrathecal administration using the iPRECIO<sup>®</sup> implanted pump

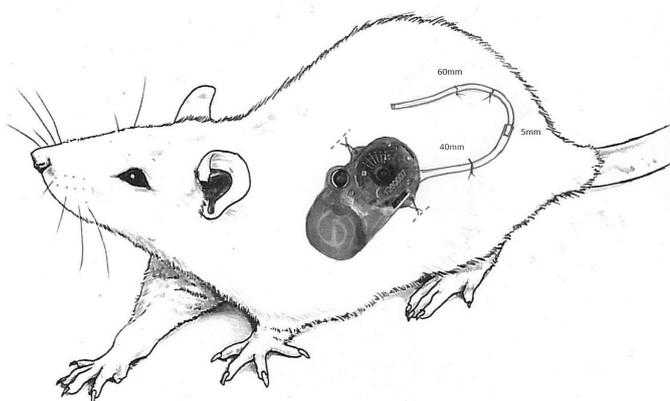
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The background to this project was a regulatory request to perform an epidural and/or intrathecal animal study to assess degradants associated with a pharmaceutical product that was given epidurally in humans. There was a concern that there might be inadvertent intrathecal administration of the product and degradants. The customer requested a rat study involving intrathecal infusion for 72-hours of two different degradant mixtures and appropriate controls, with acute and delayed toxicity endpoints and investigations of local and systemic toxicity. We had recently conducted studies involving bolus intrathecal administration to rats (100  $\mu$ L per occasion) and had veterinary surgeons on staff with the appropriate skills and experience so were confident we could help the customer meet the regulatory requirement.

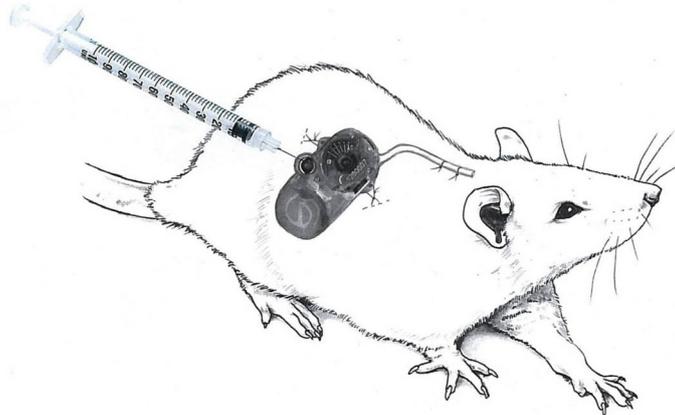


The first major challenge was the volume of dose that should be infused. The maximum human epidural dose volume equated to 200  $\mu$ L/kg/hour. The initial request was to administer up to 70  $\mu$ L/hour of degradant formulation to rats weighing 200 to 300 g (i.e. a total volume of 3 to 5 mL per rat in 72 hours). Based on total cerebrospinal fluid volume (150-300  $\mu$ l) and turnover (replaced approximately every two hours)<sup>1,2,3</sup> infusion rates of up to 3  $\mu$ l/hr have been reported as reasonable in the rat<sup>3</sup>. We located one reference that indicated 1  $\mu$ L/minute could be given to rats for four hours (240  $\mu$ L/rat in four hours) without adverse effect on sensory functions or neurohistopathology<sup>4</sup>. In considering the use of flow rates above 3  $\mu$ L/hour however, it would be important to closely monitor the animals to ensure no adverse effects were seen that

could compromise welfare, in particular for clinical signs associated with increased intrathecal pressure.

The optimal solution was to use the iPRECIO<sup>®</sup> SMP-200 programmable peristaltic pump implanted subcutaneously and linked to an intrathecal catheter

We needed an infusion system that could provide a suitable flow rate over at least 72 hours. A tethered system incorporating an exteriorised, surgically implanted catheter could have provided the required flow rate but this is not a standard method for intrathecal infusion in industry or academia and tethering would be a confounding factor in the assessment of CNS endpoints (modified Irwin assessment). Although it is possible to create variable infusion protocols (pulsatile delivery) using osmotic minipumps we decided that the optimal solution was to use the iPRECIO<sup>®</sup> SMP-200 programmable peristaltic pump<sup>5</sup> implanted subcutaneously and linked to an intrathecal catheter. This pump would allow the flexibility to start infusion immediately following surgery or at a later time. The pump has a reservoir that can be evacuated and refilled, percutaneously, by syringe and needle so there would be the opportunity for a period of recovery from surgery before administration of the degradant mixtures while avoiding the risk of catheter occlusion by administering saline or artificial cerebrospinal fluid.



## Pilot investigation and development of the surgical procedure

In the absence of definitive published information on the effects of intrathecal infusion to rats of flow rates above 3  $\mu\text{L}/\text{hour}$ , it was essential to conduct an investigative pilot study to assess the tolerability of higher flow rates. Artificial cerebrospinal fluid (CSF) was used as the infusate to replace any natural CSF removed by the infusion. Four CrI:CD(SD)<sup>®</sup> rats were used initially. A balanced anaesthetic technique was used and multimodal analgesia administered, including local anaesthetic blocks. A dorsal midline incision was made in the lumbar region and a subcutaneous pocket prepared for the SMP-200 pump. The pump was programmed and filled before being inserted into the pocket and secured with permanent suture (Nurolon<sup>™</sup> 3-0). A polyurethane catheter (PE10: ID 0.28mm OD 0.61mm) was inserted into the intrathecal space via a lumbar approach and advanced cranially before being secured to epaxial musculature with a permanent suture (Nurolon<sup>™</sup> 3-0). Confirmation of correct catheter placement was by identification of CSF flow. The iPRECIO<sup>®</sup> pump catheter and the separate, smaller gauge intrathecal catheter were connected together by inserting the latter approximately 5 mm in to the iPRECIO<sup>®</sup> catheter and sutured to underlying underlying muscle tissue. Skin closure was with surgical staples.

The pumps were programmed such that all animals initially received artificial CSF into the intrathecal space at 1  $\mu\text{L}/\text{hour}$  for seven days to allow satisfactory recovery from the surgical procedure and to monitor the effects of catheter placement. The programming was set to subsequently increase through infusion rates of 5, 10 and 15  $\mu\text{L}/\text{hour}$ , then back down to 1  $\mu\text{L}/\text{hour}$  followed by 15 and finally 30  $\mu\text{L}/\text{hour}$  (the maximum flow rate of the pump), for up to 76 hours at each flow rate, with the pump reservoir being filled with artificial CSF for this phase. This sequence of programming allowed us to monitor not only the effects of higher flow rate but also the impact of larger incremental rises in this. The response of the animals to the administered volumes of fluid was closely monitored. After a review of the findings, a further four rats were surgically prepared and pumps programmed to provide the highest tolerated flow rate (30  $\mu\text{L}/\text{hour}$ ) directly after seven days at the post-surgical volume of 1  $\mu\text{L}/\text{hour}$ , without incremental steps, in preparation for the proposed approach for the main study. Incremental rises in flow rate or direct increases from a post-recovery flow rate of 1  $\mu\text{L}/\text{hour}$  up to 30  $\mu\text{L}/\text{hour}$  resulted in no adverse clinical signs. This latter administration protocol was considered to be well tolerated and suitable for use on the main study.



## Main study

Having determined that a flow rate of 30  $\mu\text{L}/\text{hour}$  was well tolerated in the pilot study, the concentration factor for the degradants in the formulation mixtures to be administered in the main study was determined based on this infusion rate. The maximum human epidural dose of epidural anaesthetic equated approximately to a dose rate of up to 20 mL/hour. The regulatory authority cited an approximate 80-fold difference in CSF formation between rat and human and considered that rat volume rates up to 250  $\mu\text{L}/\text{hour}$  were required to achieve equivalent exposure at the anticipated levels of degradants in the stored product. It was therefore necessary to increase the concentration of degradants by a factor based on the difference in flow rates in order to attain comparable exposure with humans.

In the main study, 72 CD rats (aged 9-10 weeks and weighing 240-265 g) underwent surgery to implant the intrathecal infusion system. Surgery proceeded as described above with only minor refinements to the placement and suturing of the pump and catheter loops. The surgical procedure was carried out without major complications, with all animals catheterised successfully. CSF flow was acknowledged in all

animals except one, confirming correct placement of the catheter tip within the intrathecal space. Animals recovered well with no adverse clinical signs in the post-operative period. During the infusion at 30  $\mu\text{L}/\text{hour}$ , a small number of animals (5 out of 72) showed hindlimb paresis but at no point did this prevent the animals from moving about the cage.

### **iPRECIO® Pump handling in association with surgical implantation**

Pumps were programmed in advance of the surgery day and during this time each remained in its sterile packaging. They were warmed at approximately 45°C for at least three days prior to surgery; syringes, needles and artificial CSF were warmed from the day prior to surgery. The surgical team worked closely together to reduce the time that the pump was at ambient temperature and minimise any delays while the animals were under anaesthesia. The syringe and sheathed needle were weighed prior to filling. The pump was removed from the warming cabinet and the outside of the blister pack swabbed with 70% ethanol. After overfilling with artificial CSF through the packaging, ensuring that fluid was visibly exiting the end of the catheter, the iPRECIO® pump was activated while still contained in the blister pack. The pump was emptied and refilled with precisely 900  $\mu\text{L}$  of artificial CSF under aseptic conditions and then passed to the surgeon. Using sterile instruments the iPRECIO® catheter was cut to 60 mm (Group 1) or 40 mm (Groups 2-5) on the sterile area before surgical implantation. The intrathecal catheter was cut to size (60 mm) in advance and sterilised but not warmed.

Surgery proceeded as described earlier but with some refinements to the placement and suturing of the pump and catheter loops. Animals were allowed to recover from general anaesthesia and were then returned to the home cage when appropriate, as determined on an individual basis. Detailed surgical records were maintained for each animal, including the time of induction of anaesthesia, start and end of surgery and return to home cage, in addition to records of administration of any perioperative medication. Animals received a daily detailed inspection, with particular attention being given to the wound site and the skin overlying the iPRECIO® pump as well as general appearance and behaviour. Infusion of artificial CSF commenced on the day of implantation at a basal flow rate of 1  $\mu\text{L}/\text{hour}$ . Analgesia and antibiotics were provided as required post operatively.

### **Administration**

Artificial CSF was introduced into the pump reservoir prior to implantation. Subsequent evacuation and/

or re-filling of the reservoir within the subcutaneously implanted pump was achieved by percutaneous injection directly into the access port, which was locatable under the skin. The frequency of dose exchange was determined by the flow rate in use at the time and was required on a daily basis at infusion rates nominally above 10  $\mu\text{L}/\text{hour}$ . During the treatment period, groups of 12 male and 12 female CrI:CD(SD)® rats were treated with artificial CSF, with or without added degradant mixtures, infused at 30  $\mu\text{L}/\text{hour}$  for a minimum of 72 hours. Six males and six females were assigned to terminal assessment on Day 5 and the remainder for an 11-day recovery period to follow the 72 hours of treatment (Day 15 euthanase).

**The iPRECIO® pumps were individually calibrated by the supplier after manufacture and arrived with their own individual stated tolerance limits for accuracy. Pump flow rate accuracy was verified in a number of peer-reviewed publications.**

The iPRECIO® pumps were individually calibrated by the supplier after manufacture and arrived with their own individual stated tolerance limits for accuracy. Pump flow rate accuracy was verified in a number of peer-reviewed publications. It was therefore assumed that infusion flow rates would occur according to those programmed into the pumps before implantation. As an overall assessment of correct administration on study, the balance of the total volume of fluid introduced into all pump reservoirs for each group (and the sum of the residual fluid remaining at the end of the treatment period per group) was compared against expected dose usage. Examination of aspirated dose volumes demonstrated accurate pump function.

### **Findings related to pump implantation, intrathecal infusion and catheter connection**

A number of signs were evident that were considered to be procedural in nature or related to the presence of the iPRECIO® pump and the intrathecal catheter. Such findings included the presence of seromas in the area surrounding the pump, which were attributed to the surgical procedure; specifically the creation of a subcutaneous pocket and the presence of foreign material. Pump implantation was subsequently associated with dry abrasions and in one animal, ulceration. For two females, the presence of a seroma resulted in their premature sacrifice, as the skin had

weakened over the pump resulting in this becoming exposed. Subtle changes in motor co-ordination such as abnormal gait and slower righting reflex detected by the Irwin observation in all groups, including controls, were probably due to the relatively high intrathecal infusion rate.

It was found during the terminal necropsy procedure that the iPRECIO® catheter had become disconnected from the intrathecal catheter in a number of animals and it was not possible to categorically determine if the disconnection had occurred at necropsy or during the in-life period (prior to, during or after exposure to treatment). In most of these animals there was an absence of tissue encapsulation around the exposed catheter ends and this would suggest that separation of the catheters had occurred relatively recently, possibly during the necropsy dissection procedure.

**This method is suitable for controlled continuous infusion into the intrathecal space of the rat**

## Conclusions

This method is suitable for controlled continuous infusion into the intrathecal space of the rat. The surgical procedure is reproducible and considered to be less invasive than intrathecal access via the cisterna magna. The use of the programmable iPRECIO® pump allows for an ambulatory infusion model without the need to tether the animals. This permits behavioural assessment and is an improvement in animal welfare; animals are able to display normal behaviours post-operatively. It may also be possible for the animals to be group housed (they were singly housed on this study).

The iPRECIO® pump also allows for higher flow rates to be administered in comparison to the osmotic minipump<sup>6</sup> and infusion rates of up to 30 µL/hour are achievable. This study has demonstrated that infusion rates considerably higher than those in published data are tolerated well by the animals.

For future implants of this type further refinements would be made to the surgical procedure to ensure the model is robust. In particular improvements can be made to securing the intrathecal catheter to prevent movement, along with alterations to the connection between the intrathecal and pump catheters to improve security.

## Key points

- A study was required involving intrathecal infusion to rats for 72 hours, with acute and delayed toxicity endpoints and investigations of local and systemic toxicity
- The optimal solution was the iPRECIO® SMP-200 programmable peristaltic pump implanted subcutaneously and linked to an intrathecal catheter
- An investigative pilot study showed that incremental rises in flow rate or direct increases from a post-recovery flow rate of 1 µL/hour up to 30 µL/hour resulted in no adverse clinical signs
- Catheter disconnections were detected at necropsy in the main study for a number of animals but the absence of tissue encapsulation around the exposed catheter ends suggested this had occurred relatively recently, possibly during the necropsy dissection procedure itself

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