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Targeted Gene Therapy for CNS Disorders: Precision of Intra-Cerebroventricular (ICV) Delivery and Post-Administration Behavioral Assessments in Mice

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BACKGROUND AND PURPOSE

Gene therapy has shown significant potential for treating a wide array of genetic disorders, particularly those impacting the central nervous system (CNS). A notable delivery method in this evolving field is the intra-cerebroventricular (ICV) route, where therapeutic agents are directly administered into the cerebrospinal fluid (CSF) within the brain's ventricles. This technique offers a unique advantage by allowing treatment agents to bypass the blood-brain barrier (BBB), which typically limits the effectiveness of systemic drug delivery to the CNS. The CSF circulates through the brain and spinal cord, helping to distribute therapeutic agents across the entire CNS. Since intrathecal injection in mice is not practical, ICV is the more widely used method of choice. Although this approach holds great potential, it requires a precise and carefully executed surgical procedure to access the brain's ventricles, which carries some risks. With advances in surgical techniques, delivery systems, and gene therapy itself, ICV administration continues to pave the way for targeted treatments that could vastly improve outcomes for patients with otherwise difficult-to-treat CNS disorders.

As a standard practice in the industry, ICV injections are performed under anesthesia to minimize pain and discomfort during the procedure. However, post-procedure pain management strategies vary across the scientific literature, highlighting the need for a standardized and effective approach. This study aims to identify the most suitable post-procedure pain management method that provides adequate relief while minimizing the impact on the animal's behavior. Given that CNS study designs often include Functional Observational Battery (FOB) assessments, it is essential to ensure that control animals exhibit normal or well-characterized behavior to maintain the integrity of the study outcomes.

MATERIAL AND METHODS

The mouse has been selected as the test system as it is a model widely used in research for this procedure. At the initiation of dosing, 15 male and 15 female mice (*mus musculus*) C57BL/6, 6 to 8 weeks old, were assigned to two separate groups.

Five males and five females were assigned to the positive control group (Group 1) receiving Buprenorphine ER [extended release: 0.5 mg/mL at a dose of 1 mg/kg subcutaneously (SC)] and Meloxicam (5 mg/mL at a dose of 4 mg/kg SC) only. Animals assigned to the positive control were released to a stock colony on Day 3.

Ten males and 10 females were assigned to the ICV group (Group 2), undergoing ICV injections on Days 1 and 29 (0.9 % Sodium Chloride solution), and/or 58 (Methylene blue solution). The ICV group also received the same pain management as the positive group before ICV injection on Days 1 and 29, and a complete macro and microscopic examination was performed on Day 58.

Table 1. Study Design

Groups	Day -2	Day 1 – Procedures		Day 29 - Procedures	
Group 1 - 5M/5F	Baseline FOBs	Buprenorphine ER – 1 mg/kg Meloxicam – 5 mg/kg	FOBs at 4 and 24 hours post analgesics injection		
Group 2 - 10M/10F		**ICV injection** Buprenorphine ER – 1 mg/kg Meloxicam – 5 mg/kg	FOBs at 4 and 24 hours post ICV + analgesic injections	**ICV injection** Buprenorphine ER – 1 mg/kg Meloxicam – 5 mg/kg	FOBs at 4 and 24 hours post ICV + analgesic injections

MATERIAL AND METHODS (cont.)

While under anesthesia, the mouse was secured in a stereotaxic frame. A small midline incision in the scalp was performed to expose the skull. The bregma (a reference point on the skull) was located, and stereotaxic equipment with specific coordinates was used to inject the saline (Days 1 and 29) or methyl blue dye (Day 58). Administration (dose volume 10uL) was performed at a rate of approximately 1uL/second. The needle remained in place for a few minutes to prevent backflow of the solution, then removed slowly. The incision was sutured, and the animal was allowed to recover from anesthesia (Days 1 and 29). To confirm suitable delivery and understand potential effects on behavior post-administration, functional observational battery (FOB) was performed 4- and 24-hours post-administration to assess the animal's behavior, sensorimotor functions, and physiological responses.

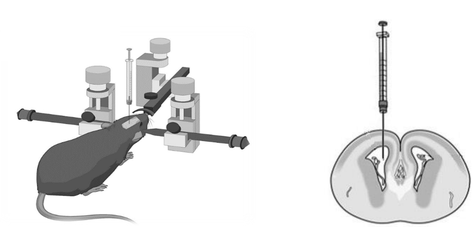

Figure 1. Intracerebroventricular Injection

Justification for Pain Management and Dose Level Selection

Buprenorphine Extended-Release (ER) is a partial μ-opioid receptor agonist commonly used in mice for prolonged analgesia. Buprenorphine ER provides extended pain relief for up to 48–72 hours after a single SC injection. The Extended-Release formulation was selected to provide long-lasting pain relief, reduce the need for frequent dosing, and minimize handling stress. A dose level of 1 mg/kg was selected to achieve a suitable analgesic effect with a potential mild sedation effect.

Meloxicam is a nonsteroidal anti-inflammatory drug (NSAID) used for pain management. Meloxicam provides analgesia for up to 24 hours after a single SC injection. The mechanism of action is a COX-2 preferential inhibitor, reducing inflammation and pain by decreasing prostaglandin production. At recommended doses, meloxicam has minimal impact on normal behavior, allowing for accurate functional observations in research studies. Therefore, a 4 mg/kg dose was selected to achieve suitable analgesia while minimizing the risk of unintended influence on behavior.

RESULTS

At 4- and/or 24 hours post-dose, animals in both groups displayed more active behavior in their home cage (**Table 2**) and the arena (**Table 3**). This was reflected by an increased incidence of the descriptor "mobile" and "moderately increased" activity when compared to the predose assessments (positive control – Day -2). Animals assessed in their home cage were noted to be more active at 4 hours compared to 24 hours post for both occasions. Similar incidences were observed on Days 1 and 29 for respective assessments and between groups.

Table 2: FOBs Home Cage—Assessment of General Behavior

		Day 1: Behavior		Day 29: Behavior	
	Day -2	4 hours post	24 hours post	4 hours post	24 hours post
Group 1 - [10 mice]	3 x mobile 4 x awake 3 x sleep	10 x mobile	3 x mobile 2 x awake 5 x sleep		
Group 2 - [20 mice]		14 x mobile 2 x awake 4 x sleep	3 x mobile 5 x awake 12 x sleep	18 x mobile 2 x awake	4 x mobile 4 x awake 12 x sleep

Table 3: FOBs Arena—General Activity and Arousal Assessments

		Day 1: Activity/Arousal		Day 29: Activity/Arousal	
	Day -2	4 hours post	24 hours post	4 hours post	24 hours post
Group 1 - [10 mice]	10 x normal	9 x normal 1 x ModIncr	5 x normal 4 x ModIncr 1 x ModDecr		
Group 2 - [20 mice]		18 x normal 2 x ModIncr	16 x normal 4 x ModIncr	16 x normal 4 x ModIncr	16 x normal 3 x ModIncr

ModIncr = Moderately Increased
ModDecr = Moderately Decreased

Unusual posture (**Table 4**) and abnormal gait (**Table 5**) (open field) were also noted for both groups, at 4- and 24-hours post-dose, on Days 1 and 29. For animals receiving the analgesic drugs alone (Group 1), observations were limited to Straub tail and tip toeing in up to 3 out of 10 animals, at 4- and 24-hours post dose.
In the ICV group, tiptoe walk and Straub tail were observed at a greater incidence than the positive control group at 4- and 24-hours post-dose. ICV animals also displayed hunched posture, indicating a more severe reaction, potentially induced by the ICV injection. In addition, on Day 29, one male demonstrated excessive swaying, and one female had her hindlimbs in an exaggerated or overcompensated drag at the 4- and 24-hour post-dose, respectively.

Table 4: FOBs Arena—Unusual Posture - Arena

		Day 1: Unusual Posture		Day 29: Unusual Posture	
	Day -2	4 hours post	24 hours post	4 hours post	24 hours post
Group 1 - [10 mice]	10 x none	8 x none 2 x straub tail	7 x none 3 x straub tail		
Group 2 - [20 mice]		9 x none 8 x straub tail 3 x hunched	12 x none 5 x straub tail 3 x hunched	6 x none 12 x straub tail 2 x hunched	4 x none 14 x straub tail 2 x hunched

Table 5: FOBs Arena—Gait Assessment - Arena

		Day 1: Gait – Arena		Day 29: Gait - Arena	
	Day -2	4 hours post	24 hours post	4 hours post	24 hours post
Group 1 - [10 mice]	10 x normal	8 x normal 2 x tiptoe	8 x normal 2 x tip toe		
Group 2 - [20 mice]		6 x normal 14 x tiptoe	9 x normal 11 x tiptoe	2 x normal 17 x tiptoe 1 x swaying	3 x normal 16 x tiptoe 1 x hind drag

DISCUSSION ON ADDITIONAL PRELIMINARY RESULTS

Since Buprenorphine ER was suspected to be the primary contributor to the increased activity levels observed post-dose, an extension of this assessment was conducted to refine the analgesic regimen. In this follow-up study, standard Buprenorphine was used, and the dose level was reduced from 1 mg/kg to 0.1 mg/kg to minimize its potential impact on activity levels while still providing adequate pain relief. Meanwhile, Meloxicam remained at a dose level of 4 mg/kg; however, to ensure sustained analgesic coverage while reducing the frequency of dosing and minimizing handling-related stress, the extended-release formulation of Meloxicam was utilized.

Preliminary findings from this modified regimen showed a reduction in the incidence of abnormal behavior within the limited sample tested, with no indications of pain or distress. These results suggest that the revised analgesic protocol may provide effective pain management while minimizing unintended behavioral alterations, thereby improving the reliability of behavioral assessments in CNS studies.

CONCLUSION

In conclusion, increased activity levels observed at 4- and 24-hours post-dose in both the ICV and positive control groups, in both home cage and open field assessments, suggest a positive effect of the selected analgesics (Buprenorphine ER and standard Meloxicam) on general behavior when compared to pre-dose baseline (positive control). However, other assessments indicated a higher incidence of unusual posture and abnormal gait in the ICV group compared to the positive control group at post-dose time points. The slightly greater severity of these observations in the ICV group suggests a potential exacerbation of the condition induced by the ICV injection.

Further refinement of the analgesic selection at a lower dose level for Buprenorphine, changing to a standard formulation for Buprenorphine, and using Meloxicam ER instead indicated very promising results. Based on these initial observations, a Phase 2 study will be initiated to evaluate further and validate this updated pain management strategy. The goal is to ensure an optimal balance between animal welfare and the integrity of behavioral assessments, ultimately refining best practices for analgesia in preclinical CNS research.

REFERENCES

990.195 -"Procedure Development for Intracerebroventricular (ICV) Dosing in Mice," February 2025.