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Intra-Cerebroventricular (ICV) Route in Mice for Administration of Gene Therapy Products

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ABSTRACT

Gene therapy (GT) has emerged as a promising approach for treating a wide array of genetic disorders and diseases. One of the innovative delivery methods in GT is the intracerebroventricular (ICV) route, which involves administering therapeutic agents directly into the cerebrospinal fluid (CSF) within the brain's ventricles. This method provides a unique opportunity to target the central nervous system (CNS) efficiently, especially for conditions that affect the brain and spinal cord. This direct injection approach can overcome the challenges posed by the blood-brain barrier (BBB), which often limits the effectiveness of systemic delivery methods. Since intrathecal injection in mice is not practical, ICV is the more widely used method of choice. To assess potential effects of the selected pain management and the injection on animal's behavior post administration, functional observational battery (FOB) assessments at 4- and 24- hours post-dose were performed, which included assessment of the behavior, sensorimotor functions, and physiological responses. The positive control group was administered Buprenorphine ER and Meloxicam, without undergoing surgical procedures. The second group underwent the surgical procedures (ICV), with 0.9% saline solution injection, and received the same pain management as the positive control group. Animals in both groups displayed a more active behavior compared to the pre-dose assessments, indicating a positive effect of the selected analgesics on the general behavior. For animals receiving the ICV injection and the analgesic drugs, activity level was comparable between 4- and 24-hours post-dose timepoint however, unusual posture and abnormal gait were noted at a higher incidence and greater severity for the ICV group compared to the positive group, indicating a possible exacerbation of the condition induced by the ICV injection. Further refinement for the analgesic selection may be necessary to mitigate the increased activity observed at 4 hours post-dose, to improve to ability to differentiate potential Test Article-related effects on the behavior at this timepoint. By overcoming the challenges posed by the BBB and achieving widespread distribution of therapeutic agents, this approach holds great potential for treating a variety of neurodegenerative diseases and genetic disorders, offering hope for improved outcomes and quality of life for patients.

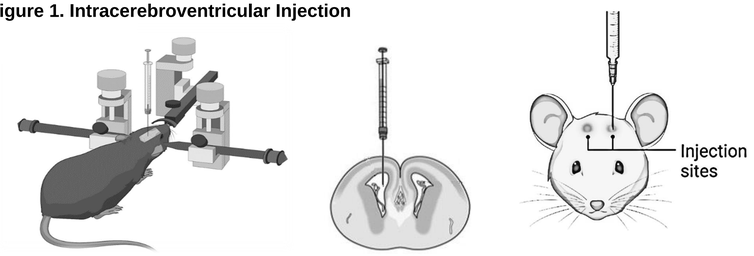
MATERIEL AND METHODS

A total of 15 male and 15 female mice (*mus musculus*) C57BL/6, 6 to 8 weeks old at initiation of dosing, were assigned to two separate groups. Five males and 5 females were assigned to the positive control group 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 5 mg/kg SC) only. 10 males and 10 females were assigned to the ICV group, 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 prior to ICV injection on Days 1 and 29 and a complete macro and microscopic examination was performed on Day 58. Animals assigned to the positive control were released to a stock colony on Day 3.

FOB assessments were performed during acclimation (5M/5F), and at 4-hrs and 24-hrs post analgesic administration (positive control) or post ICV injection and analgesic administration (ICV group). Assessments consisted of a qualitative behavior evaluation (home cage, handling, arena, and elicited responses) and quantitative assessments (body temperature).

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 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).

Figure 1. Intracerebroventricular Injection



RESULT

At 4- and/or 24-hours post-dose, animals in both groups displayed a more active behavior in their home cage (**Table 1**) and in the arena (**Table 2**). This was reflected by an increased incidence of the descriptor "mobile", and "moderately increased" activity, when compared to the pre-dose 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 incidence were observed on Days 1 and 29 for respective assessments, and between groups.

Unusual posture (**Table 3**) and abnormal gait (**Table 4**) (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 compared to 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 hours post-dose, respectively.

Table 1. 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 - M/F Total - 10 mice	3xMobile 4xAwake 3xSleep	10xMobile	3xMobile 2xAwake 5xSleep		
Group 2 - M/F Total - 20 mice		14xMobile 2xAwake 4xSleep	3xMobile 5xAwake 12xSleep	18xMobile 2xAwake	4xMobile 4xAwake 12xSleep

Table 2. 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 - M/F Total - 10 mice	10xNormal	9xNormal 1xModIncr	5xNormal 4xModIncr 1xModDecr		
Group 2 - M/F Total - 20 mice		18xNormal 2xModIncr	16xNormal 4xModIncr	16xNormal 4xModIncr	17xNormal 3xModIncr

ModIncr = Moderately Increased; ModDecr = Moderately Decreased

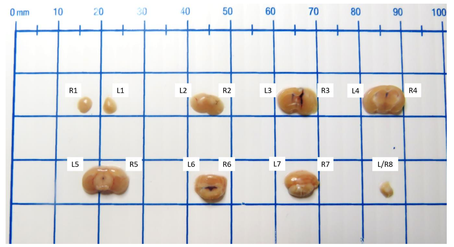
Table 3. FOBs arena–unusual posture

		Day 1: Unusual Posture		Day 29: Unusual Posture	
	Day -2	4 hours post	24 hours post	4 hours post	24 hours post
Group 1 - M/F Total - 10 mice	10xNone	8xNone 2xStraub Tail	7xNone 3xStraub Tail		
Group 2 - M/F Total - 20 mice		9xNone 8xStraub Tail 3xHunched	12xNone 5xStraub Tail 3xHunched	6xNone 12xStraub Tail 2xHunched	4xNone 14xStraub Tail 2xHunched

Table 4. FOBs arena–gait assessment

		Day 1: Gait		Day 29: Gait	
	Day -2	4 hours post	24 hours post	4 hours post	24 hours post
Group 1 - M/F Total - 10 mice	10xNormal	8xNormal 2xTipToe	8xNormal 2xTipToe		
Group 2 - M/F Total - 20 mice		6xNormal 14xTipToe	9xNormal 11xTipToe	2xNormal 17xTipToe 1xSwaying	3xNormal 16xTipToe 1xHindDrag

Figure 1. Brain sectioning



Material and Methods:
Brains from the ICV group were sectioned using a 4 mm brain matrix. Brain sections were fixed in formalin, processed to H&E slides, and evaluated microscopically.

Histopathological evaluation:
There were no remarkable microscopic findings in the brain.

CONCLUSIONS

In conclusion, higher activity level noted at 4- and 24-hours post-dose in the home cage and in the open field for both, the ICV and the positive control groups, indicated a positive effect of the selected analgesics on the general behavior when compared to pre-dose (positive control). Other assessments performed revealed that Straub tail (unusual posture) and tiptoe walk (abnormal gait) were noted at a higher incidence for the ICV group compared to the positive group at post-dose timepoints. In addition, observations in the ICV group were more severe and included hunched, swaying, and exaggerated drag of the hindlimbs, indicating a possible exacerbation of the condition induced by the ICV injection. Further refinement for the analgesic selection may be necessary to mitigate the increased activity observed at 4 hours post-dose, to improve to ability to differentiate potential Test Article-related effects on the behavior at this timepoint.

REFERENCES

990.195 - "Procedure Development for Intracerebroventricular (ICV) Dosing in Mice", October 2024.